Maria da Conceição Mesquita dos Santos
Licenciada em Engenharia do Ambiente
Mestre em Engenharia Sanitária

Effects of vegetation, seasonality, organic and nitrogen loading rates in the performance of Horizontal Subsurface Constructed Wetlands

Dissertação para obtenção do Grau de Doutor em Ambiente

Orientador: Professor Doutor António João Carvalho de Albuquerque, Professor Auxiliar, Faculdade de Engenharia da Universidade da Beira Interior

Co-orientador: Professora Doutora Leonor Miranda Monteiro do Amaral, Professora Auxiliar, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa

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FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

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"Aqueles que passam por nós, não vão sós, não nos deixam sós. Deixam um pouco de si, levam um pouco de nós.”

Antoine de Saint-Exupéry

À memória de meu marido,

Aos meus filhos, Duarte e António, fontes de inspiração, motivação e orgulho

Aqueles que preenchem o meu coração e que dão sentido à minha vida.

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RESUMO

A presente Tese pretende contribuir para o estudo de fatores críticos que influenciam a remoção de matéria orgânica e azoto em leitos de macrófitas de escoamento sub-superficial horizontal. Comprovou-se que as plantas contribuem para o aumento da remoção de matéria orgânica e azoto, porque disponibilizam oxigénio para a rizoesfera e o crescimento das raízes aumenta a área superficial para o desenvolvimento de biofilme e ainda consomem azoto e fósforo através das raízes. A *Phragmites australis* libertou mais oxigénio durante o período diurno, tendo a capacidade de transferência sido avaliada entre 0.03 e 1.53 $gO_2.m^{-2}.d^{-1}$. Observou-se uma relação linear ($p < 0.05$) entre a taxa de remoção e a carga orgânica aplicada para a carga mais baixa. A carga mássica de amónia removida não foi afetada ($p > 0.05$) pela respetiva carga aplicada.

A avaliação de desempenho de três leitos à escala real mostrou grandes flutuações na carga orgânica e de sólidos e baixa carga de azoto afluente. A eficiência média de remoção (ER) da CBO_5 foi de 88% e a de azoto foi baixa (<27% de NT, <30,8% para N-Org., <7% de NH_4^+-N e <18% de NO_X-N), embora as plantas estivessem bem desenvolvidas e o tempo de retenção hidráulico fosse adequado para promover a nitrificação. A remoção da CQO variou entre 59% e 76%. Observou-se uma relação linear significativa ($p < 0.05$) entre a carga aplicada de NT e de SST e a respetiva concentração no efluente. O efeito da variação sazonal no desempenho de outro leito à escala real mostrou que as ER dos SST e da CBO_5 não foram afetadas ($p > 0.05$) pelos períodos sazonais, enquanto as do azoto e fósforo foram influenciadas ($p < 0.05$) pela estação do ano. A ER de SST, CQO e CBO_5 não foi significativamente ($p > 0.05$) afetada pela afluência de cargas hidráulicas elevadas, o que evidencia a boa capacidade de resiliência destes sistemas.

Palavras-chave: Leitos de macrófitas, *Phragmites australis*, OTC, sazonalidade, remoção de matéria orgânica, remoção de azoto.

ABSTRACT

This thesis aims to contribute for the study of critical factors that influence the removal of organic matter and nitrogen in constructed wetlands with horizontal subsurface flow. It was realized that plants contribute for increasing the removal of organic matter and nitrogen, because they provide oxygen to the rhizosphere and the gowth of roots increases the surface area for biofilm development and uptake nitrogen through the roots. The *Phragmites australis* releases more oxygen during the daytime, and the oxygen transfer capacity was noted to change between 0.03 and 1.53 $gO_2.m^{-2}.d^{-1}$. There was a positive linear relationship ($p<0.05$) between the organic removal rate and the influent organic load for the lower load. The removal of the ammonia mass load was not affected ($p>0.05$) by the respective applied load.

The performance evaluation of three full-scale beds showed large fluctuations in organic and solid loads and low nitrogen load. The average removal efficiency (RE) of BOD5 was 88% and for nitrogen was low (<27% NT <30.8% for N-Org. <7% NH_4^+-N and <18% of NO_X-N), although the plants were well developed and the hydraulic retention time was suitable for promoting nitrification. The COD removal ranged between 59% and 76%. There was a significant linear relationship ($p<0.05$) between the applied load of NT and SST and the respective concentration in the effluent. The effect of seasonal variation in the performance of other full-scale bed has shown that the RE of TSS and BOD_5 are not affected ($p>0.05$) by seasonal periods, while nitrogen and phosphorus are significantly influenced ($p<0.05$) by the year season. The RE of TSS, COD and BOD_5 was not significantly ($p>0.05$) affected by the income of high hydraulic loads, which shows good resilience of these systems.

Keywords: Constructed wetlands, *Phragmites australis*, OTC, seasonality, organic removal, nitrogen removal.

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LIST OF ABBREVIATIONS AND ACRONYMS

BOD	Biochemical Oxygen Demand
BOD_5	Biochemical Oxygen Demand after five days
Cit.	Cited
COD	Chemical Oxygen Demand
COD_t	Total Chemical Oxygen Demand
COD_s	Soluble Chemical Oxygen Demand
CV	Coefficient of Variation
CW	Constructed Wetland
DO	Dissolved Oxygen
DOM	Dissolved Organic Mater
DP	Total Dissolved Phosphorus
DRP	Dissolved reactive phosphorus
dw	Dry weight
EC	Electrical Conductivity
EEA	European Environmental Agency
Eh	Redox potential
ET	Evapotranspiration
EU	European Union
FWS	Free Water Surface
HDPE	High Density Polyethylene
HLR	Hydraulic Loading Rate
HRT	Hydraulic Residence Time
HSSF	Horizontal Subsurface Flow
INE	Instituto Nacional de Estatística (Statistics Portugal)
IPMA	Instituto Português do Mar e da Atmosfera
IWA	International Water Association
IWRM	Integrated Water Resources Management
LECA	Light–expanded clay aggregates
NH_4^+-N	Ammonium nitrogen
NO_3^--N	Nitrate nitrogen
NO_2^--N	Nitrite nitrogen
NO_x-N	Oxidized nitrogen
NLR	Nitrogen loading rate
OLR	Organic loading rate
Org-P	Organic Phosphorus
Org-N	Organic nitrogen
ORP	Oxidation-reduction potential

OTC	Oxygen Transfer Capacity
p.e.	Population equivalent
PESAAR	Second Strategic Plan for Water Supply and Collection and Treatment of Wastewater for the period 2007-2013
PLR	Phosphorus loading rate
PNA	National Water Plan (Portugal)
PNUEA	National Program for the Efficient Use of Water
PO_4^{3-} -P	Phosphate phosphorus
POM	Particulate Organic Mater
PO_4^{3-}-P	Orthophosphate
RE	Removal efficiency
r_{COD}	Mass removal rate for COD
r_{NH4}	Mass removal rate for NH_4^+-N
ROL	Radial Oxygen Loss
SD	Standard Deviation
SF	Surface Flow
SSF	Subsurface Flow
Talk	Total alkalinity
T_KN	Total Kjeldhal Nitrogen
TP	Total phosphorus
TSS	Total Suspended Solids
UK	United Kingdom
USA	United States of America
USEPA	United State Environmental Protection Agency
VSSF	Vertical subsurface flow
WFD	Water Framework Directive
WWTP	Wastewater Treatment Plant

1. Introduction

1.1. Overview

Water is one of the most important of all natural resources. It is vital for all living organisms and major ecosystems, as well as for all aspects of human life, including public health, food production, economic development and ecosystem biodiversity, but it is a finite and vulnerable resource which has quantitative limitations and qualitative vulnerability.

The total water quantity on earth is constant and is recycled by nature in the atmosphere, on the earth's surface, below the surface and in the oceans. However, the unequal distribution of water on the planet, the exponential increase of the world population and consequent urbanization, accompanied by the increase in daily personal water consumption, rapid industrialization and the development and intensification of agriculture, have increased the demand for required quantity of water for multiple end users.

On the other hand, the combined and cumulative of many human activities has affected the water quality and make large quantities of water unsuitable for various uses, not only for human-related uses such as drinking, agricultural irrigation and industrial production but also for terrestrial and aquatic ecosystems for which clean freshwater is a prerequisite for life. Water pollution is also a serious problem for human health. Therefore, through the Agenda 21 it was recognize that freshwater resources are an essential component of earth's natural systems, indispensable for support all forms of life and it is needed in almost all human activities and therefore, the water is regarded as a structuring and strategic natural resource.

The increase in water demand, together with water pollution and climate change led to water scarcity being one of the most severe problems of the world and if the present tendency continues, by 2025 1.8 billion people will be living in countries or regions with "absolute water scarcity" (i.e., water supply less than 500 m^3/capita/annum), and two thirds of the world's population could be under "water stress" conditions (i.e., water supply less than 1700 m^3/capita/annum) (UN-Water, 2007). On the other hand, up to 2.5 million people, mostly children, die every year from diseases related to water (World Health Statistics, 2012). Also, according to information included in the "UN World Water Development Report: Water for a Sustainable World 2015" (WWAP, 2015), by 2050 the demand for water will increase by about 55%, mainly due to domestic but also to industry and demanding on electricity. Only in industrial terms, the demand increase between 2000 and 2050 is estimated that it will reach 400%. These data show the importance and urgency of implementing measures that promote the best use of available water resources.

Thus, in the beginning of 21st century, we face the hard reality that the present patterns of water consumption are not sustainable in many parts of the world and water scarcity is a well-known issue in many countries, in particular in the Mediterranean Region. Several studies indicate that southern Europe is a sensitive region to climate change, for which the aridity conditions are expected to exacerbate due to an increase in air temperature associated with a decrease in annual rainfall (Kundzewicz *et al*., 2007; Bates *et al*., 2008).

Portugal is one of those countries for which climate change regional models for the period 2080-2100 projects a substantial increase in mean air temperature all over country and an increasing frequency of droughts (Miranda, 2002). According to the same author, temperature increases will be more marked in the inner regions of the mainland (7ºC) than along the coast (3ºC). At the same time almost all models project reduction in mean precipitation and in the duration of the rainy season. Therefore, one possible consequence of these scenarios would be an overall reduction in the availability of water resources in Portugal, so it is necessary to ensure efficiency and reasonable use of this resource.

In Portugal, freshwater resources are considered abundant, despite these resources are unequally distributed in the territory, and there is great spatial, seasonal and temporal variability in rainfall which is exacerbated by climate change, consequently making flooding or drought unpredictable and at times making it difficult to sustain water flows, both in national and international rivers (APA, 2015). The precipitation is more frequent in the northern of Portugal, and generally concentrated in the winter period, mainly in the period between October and March and large variations can also occur from year to year, putting sometimes all the country in a situation of severe dryness (APA, 2015).

The general scarcity and continuous increasing pollution of freshwater resources in Portugal and in many world regions, along with the progressive encroachment of incompatible activities, demand an Integrated Water Resources Management (IWRM). So, in this context, it is therefore not surprising that the European Union (EU) on Europe 2020 agenda gives a special relevance to water as a scarce resource whose preservation is essential to ensuring effective sustainable development of all regions. In achieving this goal there are two main sets of measures: one, aimed at the efficient use of water; another centered on the concern to ensure the surface freshwater and groundwater meet minimum quality standards. In fact, one of the current concerns about the water issue is linked to the efficient management of this resource in order to preserve or improve the physical, chemical and biological quality of water bodies, and consequently, it is essential to implement coherent and sustainable policies.

The international strategies and recommendations on integrated water management defined in the Lisbon Charter (IWA , 2015) and the United Nations Millennium Development Goals (IED, 2010) pointed out the need of good practices in wastewater services in order to improve the quality of water resources and to better protect the public health. The Millennium Development Goals on environmental sustainability suggest the use of low cost and ecological systems for improving the population served with sanitation facilities and good quality water sources (IED, 2010), being necessary analyzing the influence of climate variation and the performance of wastewater treatment devices.

The Water Framework Directive (WFD) (Directive 2000/60/EC), transposed in Portugal by Law No. 58/2005 of December 29 (Water Law), modified by Decree-Law No. 130/2012 of 22 June, was a step forward in the European water resource policies that sets out, for the first time, a detailed and integrated framework for the improved protection and restored clean water resources and aquatic environments across Europe and ensures its long-term and sustainable use. Under this directive, the European member states will have to ensure that "good status" is achieved or kept in all waters by 2015.

In parallel with the Water Law, there is still a set of normative documents that are key instruments of national policy to define and support the guidelines for improve environmental conditions on water resources and the efficient use of water, in order to harmonize their uses and their availability, such as the National Water Plan (PNA) (Decree-Law No. 112/2002 of 17 April, modified in part by No. 130 Decree-Law/2012 of 22 June) (APA, 2015) and programs such as the National Programme for the Efficient Use of Water (PNUEA) (MAOT, 2001; APA, 2012), which will be implemented in the period 2012-2020.

Urban wastewater and agriculture activities are the main sources of water pollution throughout the world (Elnabousi, 2011) and therefore, the wastewater treatment has become one of the most important environmental issues due to its importance on keep and increases the quality of the water natural resources. In fact, wastewater discharged contains several waterborne pollutants, which contributes to a variety of water pollution problems. Domestic wastewater contains relatively high concentrations of organic matter and nitrogenous compounds, which are important examples of those pollutants, and represent a predominant point source of nitrogen pollution to surface waters (Dzakpasu *et al.*, 2011).

In Europe, nutrient discharges from municipal wastewater treatment plants are in general higher than from any other point source. Results from large inland and marine catchments show that municipal wastewater constitutes about 75 % of the point source discharges of both nitrogen and phosphorus, while industrial sources constitute about 17 % and other point sources are relatively insignificant (EEA, 2012). These pollutants can cause environmental deterioration just like eutrophication, which can stimulate the growth of algae, increased water purification cost, interference with the recreational value of water, health risks to public health, and can lead to decreased dissolved oxygen levels and undesirable changes in aquatic populations (Wang *et al*., 2007; Song *et al.*, 2010; Chen *et al.*, 2011).

So, in order to diminish the overall impacts of these effluents in receiving water bodies, there is the need for careful planning, adequate and suitable treatment before discharge, regular monitoring and appropriate legislation, which are becoming more stringent and in the future, can become even more stringent. This can be achieved through the application of appropriate wastewaters treatment processes, which will help to minimize the risks to public health and ensure the sustainability of the environment, ensuring that the release of effluents into water bodies will not cause changes in its quality, by meeting not only the effluents standards, but also the limits set in License Use of Water Resources for Water Discharge Waste.

An important obligation concerning wastewater handling is the European Directive 91/271/EEC that demanded the construction and functioning of a wastewater treatment plants (WWTPs) with secondary treatment for all agglomerations of more than 2000 inhabitants, by the year of 2015. In Portugal, this directive was implemented by the Decree-Law No. 152/97 from 19 July (altered by Decree-Law No. 172/2001 from 26 May and later revised by Decree-Law No. 149/2004 from 22 July).

Therefore, to achieve a “good status” in all water bodies, it is of major importance to improve not only the sanitation systems of larger cities, but also to upgrade the sewage handling in smaller communities. The Portuguese Governments have made significant investments over recent years in infrastructures to protect and enhance water quality, but more effort was required in order to achieve our goals under the WFD by 2015. One of the objectives of the Second Strategic Plan for Water Supply and Collection and Treatment of Wastewater for the period 2007-2013 (PEAASAR II) was to provide 90% of the country’s total population with public urban wastewater treatment systems in 2013 (MAOTDR, 2007).

However, according to the new strategic plan that was formulated in 2015 and known as PENSAAR 2020 (MAOTE, 2015), the levels of service of wastewater collection and treatment systems were still below established targets, with the sewage connection rate at 81% of the population, and sewage treatment plant connection at 78% in 2011. There are still important regional differences, mainly in interior regions, where the needs for providing suitable treatment for the effluents of small rural communities continue to be pertinent and the service levels is significantly lower than the national average, especially in the northern and central regions (INAG, 2011).

PENSAAR 2020 defined the new strategy for the water and wastewater sector in Portugal that is less centered in new infrastructure and focuses more on the management of the sector assets, its operation and the quality of the services provided. The performance of wastewater treatment systems should ensure environmental protection and contribute to improving the quality of water bodies, taking into account not only legal compliance imposed by discharge regulations, reduction of the urban pollution of water bodies as well as the sustainable use of natural resources, and thus contribute to minimize environmental impacts and reduce operating costs whilst improving the environmental and economic performance of the systems, another of the strategic objectives of the new plan for the sector.

Thus, one of the challenges is the rural areas of Portugal, that are inhabited by approximately 39% of the country’s population, and they are often characterized by widely dispersed agglomerations, with small populations (< 2000 inhabitants), which do not benefit from the economies of scale that large systems have (INE, 2012).

In Portugal, the Decree-Law No. 152/97 from 19 July referred that is required an *“appropriate treatment*” for wastewater for populations lower than 2000 inhabitants, which means a treatment level that ensure the quality standards (established by Portuguese Decree-Law No. 236/1998 from 1 August) for protect the aquatic environment and that also contribute for to increase the quality of receiving water bodies for support its principal uses. In spite of communities with less than 2000 inhabitants not having any European legally requirement obligation regarding sewage treatment, to achieve a good water status in all water bodies it would be difficult to accomplish without improving an adequate sewage system in the rural areas.

In Portugal, the usual wastewater treatment techniques applied to small agglomerations include conventional systems such extended aeration activated sludge and trickling filters, septic tanks and, in some cases Imhoff tanks. Septic systems, also known as “on-site wastewater treatment systems,” are widely used in rural settings to dispose of wastewater in Portugal. Operating properly, these systems, removes many pollutants and provide some measure of protection for human health and for the environment. However, as aquifers and water bodies tend to exhaust, especially at summer period, its water quality must be progressively declining and thus the level of treatment achieved by the septic systems may not be sufficient for maintaining the "good status" in water bodies.

So, for the selections of sanitation technologies of small wastewater treatment systems, Portuguese PENSAAR 2020 establish, besides the strategic objectives of the universality, continuity, quality of service and protection of human health and environmental values, the necessity to achieve solutions which ensure economic, operational and environmental sustainability of those systems. The reuse of treated wastewater should also be seen as a part of the solution for ensuring good governance of water resources.

The wastewater treatment solution in this context requires decentralized, innovative and sustainable approaches that are appropriate for minimize the risks to public health and protecting water resources from pollution and should also ensure minimal costs of construction, operation and maintenance. At present, most researchers are focused on environmental friendly treatment technology, simple to operate, less energy-intensive and cost-effective. These technologies, also called as non-conventional technologies, are those that achieve the elimination of pollutants from wastewater through natural processes and mechanisms that do not require external energy or chemical additives and that depend on the sun, air temperature, microbial life, soil or plants. These natural techniques have been increasingly used due the low-cost, little maintenance and some of them; also have the advantage of do not generate sludge as with conventional treatment systems (Kreissel, 2007; Garcia *et al*., 2010). However, generally these technologies are less compact than conventional WWTPs and require a larger treatment surface area (Garcia & Corzo, 2008).

Several alternatives for small agglomerations have been recently studied and developed and one of the examples of these natural and environmentally friendly systems is the constructed wetlands

(CWs), which is one of the non-conventional techniques that became more popular and effective for removal various pollutants present in different categories of wastewater and at wide variety of climatic conditions worldwide (Rousseau *et al.*, 2004; Wallace & Knight, 2006; Cooper, 2009; Kadlec & Wallace, 2009; Dan *et al.*, 2011).

CWs are engineered systems that have been designed and constructed to create ecological condition similar to natural wetlands, involving aquatic macrophytes plants, soil and their associated microbial community, which are responsible for wastewater treatment (Kadlec & Knight, 1996). These systems require low anthropogenic energy and chemical inputs and low skilled labor for operation when compared to the conventional treatment systems (Konnerup *et al.*, 2009; Yu *et al.*, 2012). Therefore, these natural systems may be especially effective and practically feasible in rural and low-density areas, as is the case of interior regions of Portugal.

The use of CWs for wastewater treatment is becoming more and more accepted and has gained popularity in many parts of the world including developing as well as developed countries (Garcia *et al.*, 2010). Portugal is not an exception and during the last twenty years, there is a tendency, mainly in the Central Region of the country, for the construction of this type of technology, in particularly Horizontal Subsurface Flow Constructed Wetlands (HSSF-CWs). These systems are a relatively new technology, and although considerable numbers of studies have contributed to understanding of the physical and biochemical reactions that promote the pollutant removal process, inconsistence results suggest that further studies are required to better understand these complex ecosystems for optimizing system design and operation.

In fact, many studies have shown that CWs significantly reduce concentrations of pollutants like, organic matter, heavy metals, suspended matter and pathogens (Fisher & Acreman 2004; Vidales-Contreras *et al.*, 2006; Vymazal, 2007; Reinoso *et al.*, 2008; Kadlec & Wallace 2009; Zhang et al. 2011). However, nutrient removal (nitrogen and phosphorus) seems to be more variable due to the complex interaction of several parameters such as water chemistry, climate (air temperature, solar radiation and precipitation), pollutant concentrations and vegetation, each of which has its own annual cycle, causing changes in nutrient supply, uptake or release of chemical substances and biological activities of microorganisms and plants (Passeport *et al.*, 2009; Zhang *et al.*, 2011; Saeed & Sun, 2012). In fact, while some studies report removal efficiencies up to 90% for total nitrogen (TN), other studies show very limited reduction, even lower as 11% or even addition of nitrogen (Nivala *et al.*, 2007; Vymazal, 2007). Concerning to nitrogen removal in HSSF-CWs, typical removal efficiencies of ammoniacal-nitrogen (NH_4^+-N) in European systems ranged between 30-40% and 40 % of TN (Vymazal, 2005).

The roles played by plants in these treatment systems are still lacking, especially little research has been do about on its oxygen capacity transfer, which are essential for the nitrogen removal processes, given that most studies reported that nitrification followed by the denitrification was a mainly method of nitrogen removal in CWs (Cui *et al.*, 2010; Ding *et al.*, 2012; He *et al*., 2012). In fact, many studies show that, a wetland system with vegetation has a higher efficiency of pollutant removal than that without plants (Kaseva, 2004; Riley *et al.,* 2005; Akratos & Tsihrintzis, 2007; Bwire *et al.* 2011) while others did not detect any significant difference between planted and unplanted systems (Baldizon *et al.* 2002).

Seasonal variations on CWs performance are also reported by several researchers (Rousseau *et al*., 2004; Davison *et al.*, 2005; Garbett, 2005; Picard *et al.*, 2005; Dušek *et al.*, 2008). For example, temperature seems to play an important role in nitrification and denitrification microorganisms in CWs (Garcia *et al.*, 2010; He *et al*., 2012; Saeed & Sun, 2012; Tao *et al.*, 2012). According to Kadlec & Knight (1996), the optimum temperature for these biochemical processes ranges from 20 to 25 ºC, and it seems there is almost not nitrogen removal to values lower than 6 ºC (Abbasi *et al*., 2016).

So, the CWs systems in winter can present lower nitrogen removal rates due to the lower temperatures, especially in some interior areas of North and Central of Portugal, where winter mean values of minimum temperature can be less than 2 °C (IPMA, 2013a). In addition, most plant are dormant and wilting in winter, and thus contributing to an oxygenation deficiency in the root zone, which is a key factor for the process of nitrification to take place, leading to reduce the nitrogen removal capacity of CWs.

Although the use of these systems has been widespread, there is still a lack of knowledge of the full removal pathways for some contaminants and so, it is very important to understand the treatment variability and the factors that can influence the interactions and complex reactions that could take place in the wetlands. On the other hand, for management purposes, it is also imperative to monitor the full-scale systems to better understand its functioning, especially in Portugal where studies are still limited. In particular, a better understanding of the biochemical transformations occurring in HSSF-CWs, and the variables that affects the cycling of pollutants is needed in order to design systems that can meet the environmental guidelines.

1.2. Research objectives

CWs are a wastewater treatment technology that has been introduced in Portugal in the last 15 years and studies on these systems are lacking. Since the HSSF-CWs is the dominant CW in Portugal, the present study is focused on this type of bed and the overall objective of the research was to contribute for the understanding and the evaluation of the effects of some critical parameters in the effectiveness of the HSSF-CWs, such as loading rate, vegetation, and season. The research is intended to help provide a more consistent basis for optimizing system design and operation, and to develop strategies to reduce any unwanted effects, promoting the ability of these systems for pollutant removal.

With this perspective, at the laboratory and full scales, HSSF-CW systems were investigated for evaluate their capacity for removing organic matter and nitrogen compounds from wastewater.

Since, the HSSF-CWs is the most common type CW in use in Portugal, the treatment performance of full-scale systems of the central region of Portugal was evaluated in order to determine their effectiveness in removing pollutant, especially organic matter and nitrogen compounds and to understand the removal mechanisms occurring within full-scale wetlands systems. This study also aimed to investigate and compare the annual and seasonal removal efficiencies.

The study was also conducted for evaluating the effects of plants in treating wastewater through the comparison between unplanted and planted HSSF-CWs at laboratory scale. Additionally, it was also evaluate the impact of mass loading of ammonia-nitrogen and chemical oxygen demand (COD) on organic matter and nitrogen removal processes.

Available oxygen in CWs is an important factor for the degradation of organic matter and on biochemical transformations of nitrogen compounds, both of which are oxygen limiting processes. However, the role of plants for oxygenation of rhizosphere still remains unclear and there are limited studies that have quantified the oxygen transfer capacity of macrophytes. Therefore, a study was conducted for evaluating the oxygen transfer capacity by the roots of *Phragmites australis*, the most common wetland plant used in Portugal. The purpose of this part of the research was also to determine whether oxygen release through the roots could be important for enhacing aerobic and anoxic removal mechanisms in the rizhosphere.

In order to meet these objectives, a number of experiments were carried out both in field and under laboratory conditions. The field studies were conducted at four CWs located in the Interior Central Region of Portugal at villages of Aranhas, Capinha, Janeiro de Cima and Sarnadas de Rodão. The region is characterized by a climate temperate Mediterranean, with marked continental effect, with high temperature ranges and almost all of the rainfall concentrated in autumn/winter (Costa, 2006; IPMA, 2013a; IPMA 2013b). The laboratory experiments were carried out at the Laboratory of Environmental Sanitation of the Department of Civil Engineering and Architecture of the University of Beira Interior.

1.3. Thesis outline

This dissertation is structured in 7 chapters each focusing on a specific topic and an appendix. (Figure 1.1).

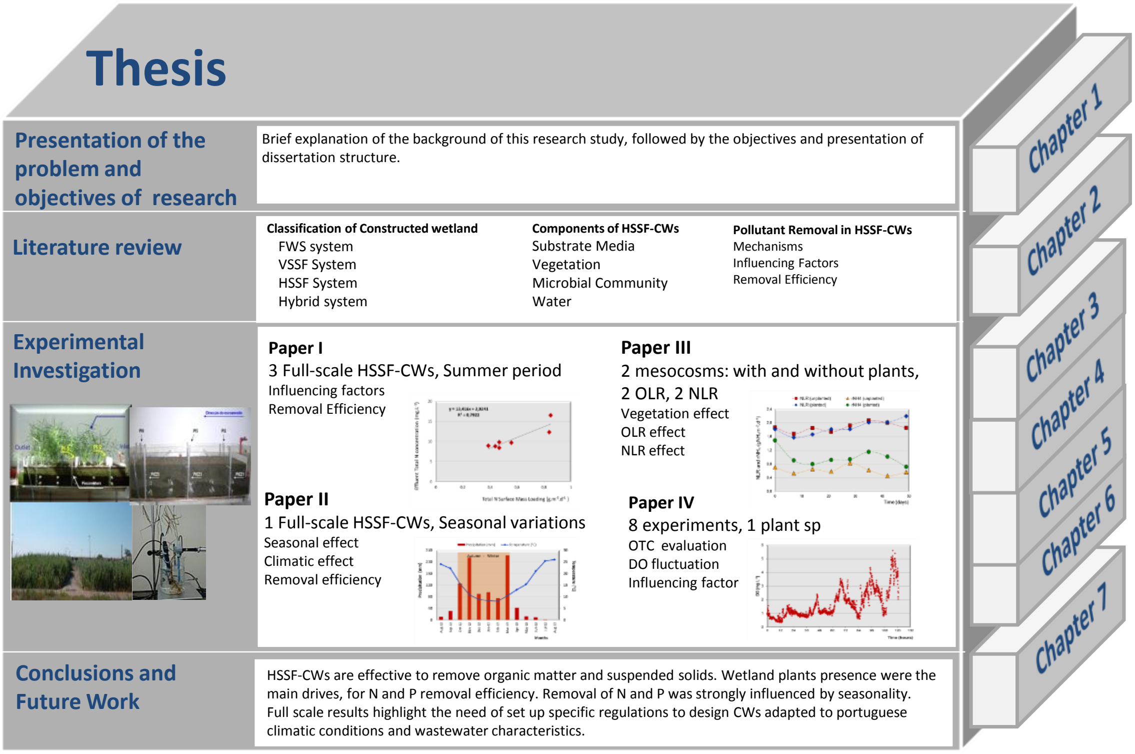

Figure 1.1 Thesis structure

Introductory chapter, Chapter 1, consists of a brief explanation of the background of this research study, followed by the objectives and presentation of the description of the organization of the dissertation, while chapter 2 presents the outcomes of the critical review of research literature on CWs in order to provide documented support and outlook to subsequent chapters, along with a historical background on the subject. An overview is given of the CWs referring the different typology of flow systems (surface flow, subsurface flow and hybrid types), the role of main wetland components and enumerating the different removal mechanisms of pollutants. The review will also describe the critical factors that influence the potential removal of pollutants, the efficiency of HSSF-CWs to treat domestic wastewater and current best practices to enhance its performance.

Chapters 3, 4, 5 and 6 are written in a publication format and include the introduction, methods, results and discussions associated with each one. Chapter 3 presents the assessment of the wastewater performance of the HSSF-CWs treating primary effluent of domestic wastewater under Central region conditions of Portugal. This chapter discusses the methodology, findings and conclusions of the field study conducted on three full-scale HSSF-CWs for determine the removal of organic matter (COD and BOD_5), suspended solids and nutrients at summer period. The summer period was chosen because is the season that may present more problems for CW performance due to high evaporation rates and high variation of incoming loads related to the presence of a seasonal population that significantly increases in the zone studied during the summer holidays and that is a common issue in rural areas of Portugal interior region. The paper also discusses the relationships between loading rates and removal efficiency (RE) and effluent concentrations in order to better clarify those results.

Chapter 4 discusses the seasonal variability and the influence of climatic conditions in the performance efficiency of the one full-scale HSSF wetland system located in a Continental-Mediterranean Climate Region at Interior Centre of Portugal. This paper outlines the influence of temperature, flow rate and input concentrations on the contaminant removal (organic matter, suspended solids and nutrients) in CWs.

Chapter 5 provides a description of the laboratory study that addresses objectives related with the effect of vegetation (*Phragmites australis*) on removal processes. The planted mesocosm scale HSSF-CW system was compared to the unplanted one in order to elucidate the role of the plants on the removal of the organic matter and ammonium-nitrogen. The effects of pollutant mass load (organic and ammonium-nitrogen loading) on their respective removal processes was also evaluated. Two loads of synthetic effluent with one plant conditions (*Phragmites australis*) and an unplanted control were tested simultaneously.

Chapter 6 provides details on the laboratory investigation carried out to evaluate the contribution of wetland plants for oxygenation of the root zone, an issue that remains controversial, and so to provide a better understanding of the role of vegetation in wastewater treatment in CWs. The objective of this study was to calculate the oxygen transfer capacity (OTC) of the *Phragmites australis,* which is the most common plant used in HSSF-CWs in Portugal.

Chapter 7 compares and discusses the results from the different chapters and provides the main conclusions derived from the study. Recommendations for future research are also presented.

The thesis appendix presents the list of papers and posters that were published or presented on conferences related to this work.

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2. Horizontal Subsurface Flow Constructed Wetlands for wastewater treatment: a review

2.1. Introduction to wetlands

According to the *International Ramsar Convention on Wetlands* (1971) (Article1.1), *wetlands systems* are viewed as "areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt, including areas of marine water the depth of which at low tide does not exceed six metres" (Ramsar, 2012). On the other hand, *natural wetlands* are considered "transitional lands between terrestrial and aquatic eco-systems where the water table is usually at or near the surface or the land is covered by shallow water" (Scholz & Lee, 2005). These natural systems are habitats of great ecological value which allow developing aquatic ecosystem biodiversity of surface water and assume an important role in the global water cycle, as they provide, for example, flood control, storage of storm and runoff waters and water purification, as well as participating in erosion control (Mitsch & Gosselink, 2000; Zhang *et al.* 2010). They are also seen as ecosystems of primary importance for nutrient balance in their surrounding environments, for groundwater recharge and carbon sequestration.
Constructed wetlands (CWs) are man-made artificially wetlands, built in areas where wetland ecosystems do not naturally occur, and include wetlands which are built for different purposes such as wastewater treatment, water storage and drainage ditches, salt exploitation, seasonally flooded agricultural land, aquaculture activities (fish and shrimp), and irrigated agricultural lands (rice paddy fields) (Ramsar, 2012). In the field of wastewater treatment, "constructed wetlands" are biological wastewater treatment technology, which use the advantages of natural wetlands for removing pollutants in a more controlled environment (Sundaravadivel & Vigneswaran, 2001). Its capacity for acting as "living filters", improving the quality of water, has for long been recognized (Mitsch & Gosselink, 2000; Sundaravadivel & Vigneswaran, 2001; Vymazal & Kröpfelová, 2008). There are records on wetland systems for receiving wastewater since 1912 (Kadlec & Knight, 1996), and it is believed that similar systems where used by the Egyptians and Chinese to clarify effluent at thousands years ago (Wallace & Knight, 2006).

The first research studies on wetlands system for wastewater treatment seems to have occurred in Europe during the 1950s with the experiments carried out by Käthe Seidel in 1952 at the Max Planck Institute in Plön, Germany (Cooper *et al.*, 1996; Kadlec & Knight, 1996; Vymazal *et al.*, 1998). Later, at the beginning of the 60s, Reinhold Kickuth, who was a student of Käthe Seidel, of the university of Göttingen (Germany), developed a wetland treatment process which became known as the *Root Zone Method*, which was used for the first operational full-scale CW built in Europe, in Othfresen (Germany) for the treatment of municipal wastewaters (Brix, 1994; Vymazal & Kröpfelová, 2008). At the same time, by 1960, research studies on artificial wetlands systems were also conducted by the United State Environmental Protection Agency (USEPA), United State Army Corps of Engineers, National Science Foundation and United State Department of Agriculture, and the first full-scale systems were installed in the USA in the 1970s with an increasing number in the 1980s (Kadlec & Knight, 1996). According to the literature data, nowadays there might be more than 50 000 CWs in Europe and more than 10 000 in North America (Kadlec & Wallace, 2009; Yan & Xu, 2014).

The application of CWs for wastewater treatment has been used for over 50 years, although in the last 25 years has increased considerably the number of these systems (Zhang *et al.*, 2010). Nowadays this technology is widespread over the world, and is recognized as a reliable and suitable secondary treatment technology for many types of wastewaters such as domestic, municipal wastewater, industrial and agricultural wastewaters, acid mine drainage and also for landfill leachate or as tertiary treatment for polishing wastewater (Calheiros *et al*., 2009; Isosaari *et al*., 2010; Pedrero *et al*., 2011; Amado *et al*., 2012; Bialowiec *et al*., 2012).

CWs are an attached-growth biological treatment technology, which uses numerous interdependent and symbiotic processes for simultaneous removal of different types of pollutants such as sedimentation, filtration, precipitation, volatilization, adsorption to substrate media and biofilm, assimilation by the plants tissue and microbial degradation. These sytems are designed for working as natural wetlands, although with a higher degree of control and are characterized by having a higher rate of biological activity, where the most common pollutants presented in wastewaters can be converted into harmless by-products and/or important nutrients that can be used both to increase their own biological productivity as to be recycled through the use of effluent in irrigation of agricultural land (Carty *et al.*, 2008; Marecos do Monte & Albuquerque, 2010).

This technology seems to have several advantages over the conventional wastewater treatment technologies such as the ability to support variable hydraulic and/or pollutant loading and as already noted, relatively low construction and maintenance costs. Besides, they can use local materials and labour and requires an operator during less time both in terms of operational and maintenance needs (Scholz & Lee, 2005; Vymazal, 2010). Additionaly, the use of these systems can also provide a particular value of flooding control, the opportunities to create or restore wetland habitat for wildlife and they help to enhance the landscape aesthetically (Sundaravadivel & Vigneswaran, 2001; Katsenovich *et al.*, 2009; Vymazal, 2011).

However, there are also some disadvantages linked to the use of these systems such as the need of large land areas comparing to conventional treatment technologies, what is due to relatively slower rate of process. Their performance may also be less consistent than in conventional treatment, because CWs treatment depend on climate and, thus, may have reduce efficiencies during colder seasons and their effectiveness seems also susceptible to heavy rains and floods (Mena Sanz *et al.*, 2007; Vymazal, 2013).

2.2. Types of Constructed Wetlands

CWs are classified according to several criteria, but the most common are the direction of water flow regime and the type of dominating macrophytes growth (Kadlec & Knight, 1996; IWA, 2000; Vymazal, 2009).In terms of the hydraulic regime, the CWs could be classified into two major types (Fonder & Headly; 2010):

i) Surface flow wetlands (SF), often referred to free water surface constructed wetlands (FWS-CWs);
ii) Subsurface flow wetlands (SSF) Constructed wetlands.

Each of these systems can be further subdivided into several variants (Figure 2.1).

Not all wetlands vegetation is suitable for wastewater treatment because in treatment wetlands plants must be able to tolerate variable concentrations of pollutants (IWA, 2000). According to the life form of the dominant macrophytes in the wetland, systems can be also classified as (Figure 2.2):

i) *Emergent macrophytes* species systems that use plants rooted in the substrate media but whose stems, leaves and reproductive organs are above the water level. Generally they are herbaceous (*e.g*., (a) *Scirpus lacustris*; (b) *Phragmites australis*; (c) *Typha latifolia* and *Juncus* spp.), however there are also some woody species such as *Taxodium distichum*, *Salix* and *Populus* genera;
ii) *Floating leaved macrophytes* species systems, also known as floating attached plants, due to its roots that are anchored in the substrate while leaves are floating on the water's surface (e.g., (d) *Nymphaea alba*; (e) *Potamogeton gramineus*; (f) *Hydrocotyle vulgaris*;
iii) *Free-floating macrophytes* species systems, plants which have leaves and stems freely floating on water surface and that move on the water's surface by winds and water currents (e.g., (g) *Eichhornia crassipes* (water hyacinth); (h) *Lemna minor* (duckweed); *Pistia stratiotes* (water lettuce);
iv) *Submerged macrophytes* species-based systems that include rooted plants with most of their vegetative mass below the water surface (e.g., (i) *Potamogeton crispus*; (j) *Littorella uniflora.*

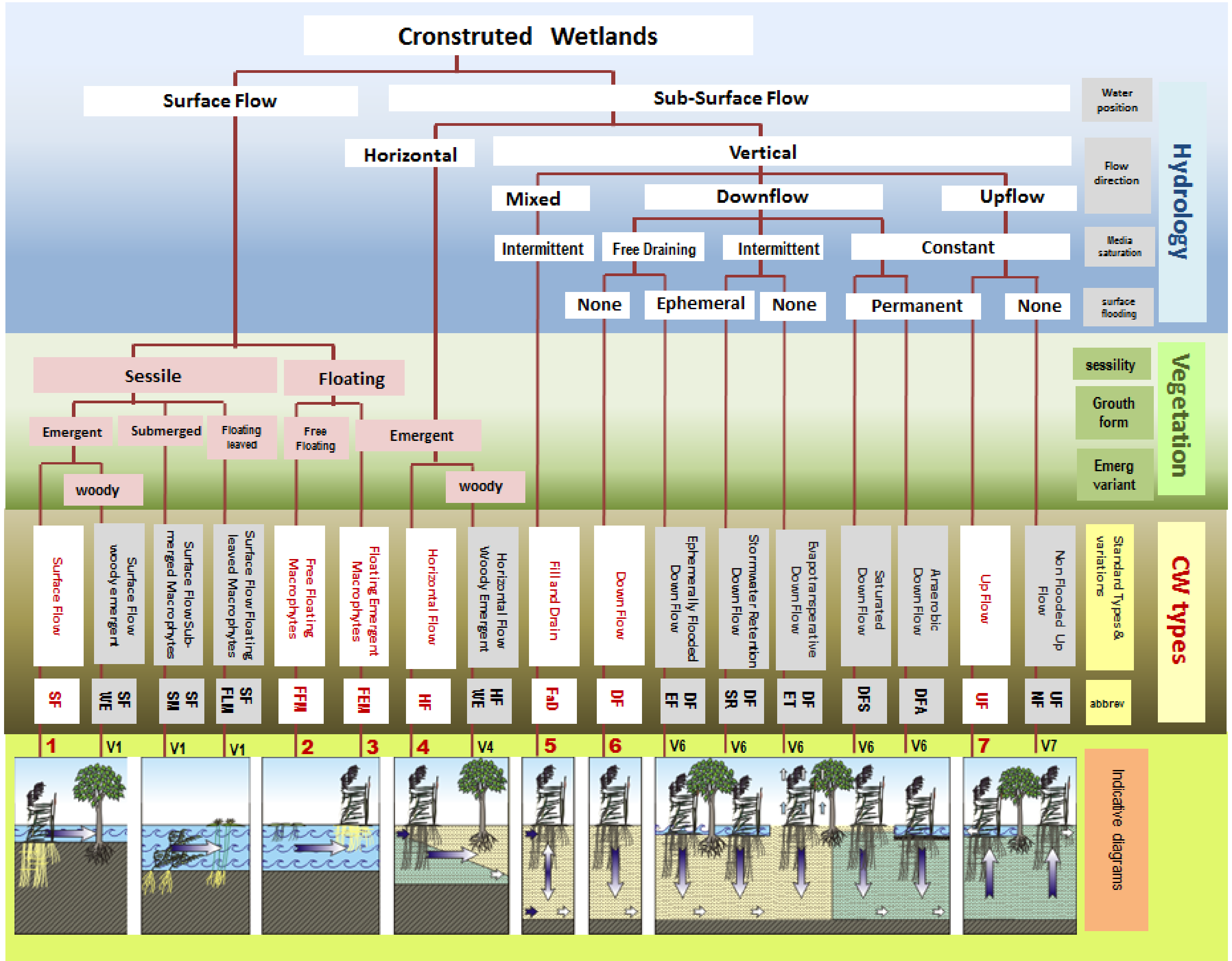

Figure 2.1 Constructed Wetland Classification System according to Fonder & Headly (adapted from Fonder & Headly, 2010).

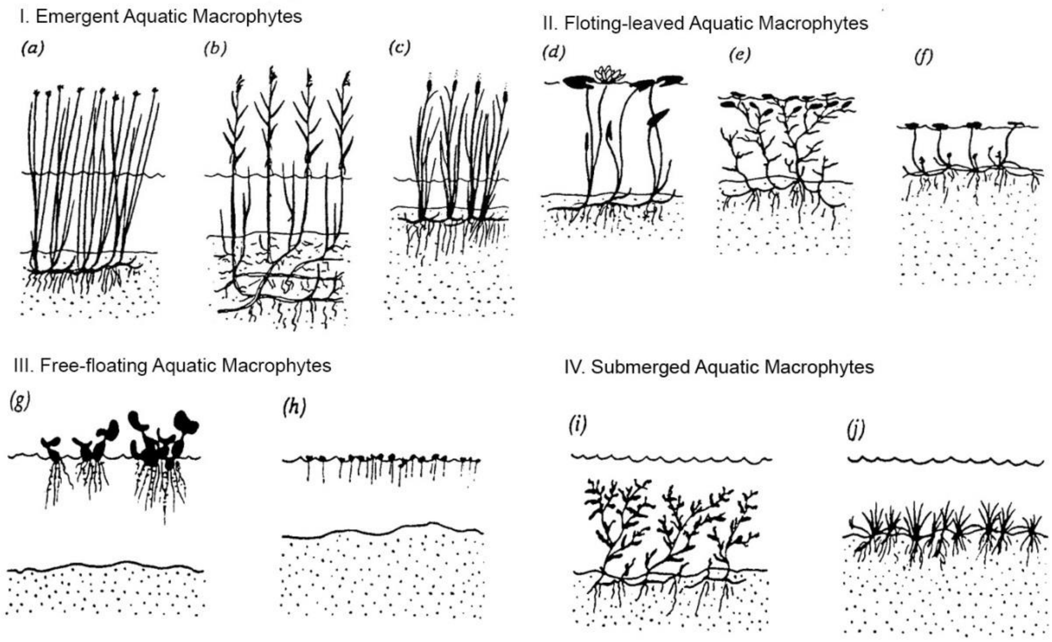

Figure 2.2 Classification of aquatic macrophytes based on growth form (Adapted from Brix & Schierup, 1989 in Vymazal *et al*., 1998)

2.2.1. Free water surface flow systems (FWS-CWs)

The free water surface flow systems seem to be the older technology and consist of a shallow basin or sequence of basins or channels, filled with 20-30 cm of suitable substrate to support the rooted vegetation when these are present, with a layer of water with a depth ranges from < 0.1 m up to 0.8 m, depending on the purpose of treatment, wastewater characteristics and climatic conditions (Kadlec & Knight, 1996; Vymazal, 2010). The inlet and outlet structures are placed at the same level (Brix, 1994). The first full-scale FWS-CW was built in Netherlands to treat wastewaters from a camping site during the period 1967-1969 (Vymazal, 2005).

At the bottom of the wetland system, an impermeable barrier (liner or native soil) is essential to avoid infiltration, and consequently, contamination of groundwater, a standard practice for all the types of CWs treatment systems. In these systems, the wastewater flows horizontally above the surface of the bed, similar of most natural wetlands and exposed to the atmosphere (Figure 2.3). For that reason, there is a high probability of human exposure to pathogens, and therefore, these systems are generally only used for polishing effluent from secondary treatment processes (Vymazal, 2010).

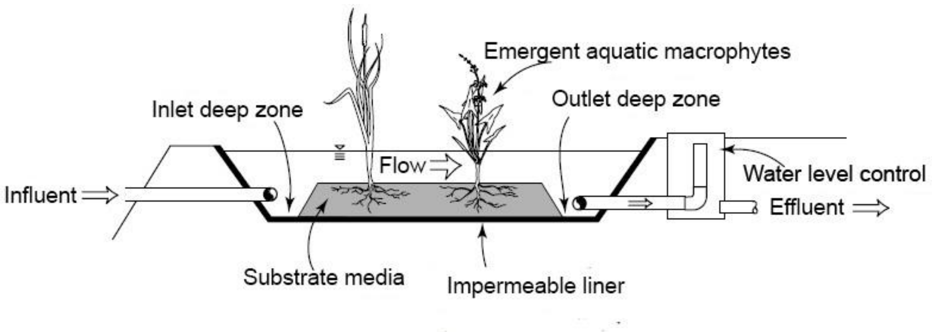

Figure 2.3 Schematic representations of a surface flow wetland system with emergent plants (Kadlec & Wallace, 2009).

During colder seasons FWS-CWs system can be operated with increased depths to offset the presence of an ice cover which will decrease the hydraulic residence time due to volume reduction (Kadlec & Wallace, 2009). According to Kadlec & Knight (1996) the area can be calculated as a rule of thumb with a 5-10 m^2/p.e. required for secondary treatment and a length-to-with ratio of 2 or higher is purposed, in order to promote plug-flow regime and to reduce short-circuiting. The hydraulic retention time (HRT) should be between 5-14 days, depending on the type of wastewater and climate. The same authors suggest the use of an organic loading rate (OLR) lower than 80 kg $BOD_5.ha^{-1}day^{-1}$ (8 g $BOD_5.m^{-2}.day^{-1}$).

Several species of wetland plants have been used in this type of CWs. The principal floating species used in these systems are water hyacinth, duckweed and water lettuce, while the floating-leaved species most common used are water lilies (*Nymphaea* spp.), lotus (*Nelumbo* spp.) and lily (*Nuphar* spp.) (IWA, 2000). Submerged aquatic plants such as waterweed (*Elodea* spp.), water milfoil (*Myriophyllum* spp.) and naiads (*Najas* spp.) are examples of plants that also have sometimes been used to treat wastewater in FWS systems (Vymazal, 2010). However, according with the last author, the most frequently species used in FWS-CWs are emergent plants, especially persistent emergent plants, such as bulrushes (*Scripus* spp.), spikerush (*Eleocharis* spp.), sedges (*Cyperus* spp. and *Carex* spp.), rushes (*Juncus* spp.), common reed (*Phragmites australis*), reed canary grass (*Phalaris arundinacea*), sweet mannagrass (*Glyceria maxima*) and cattails (*Typha* spp.). Vymazal (2011) stated that plants were usually planted at densities of around 10 -15 plants.m^{-2}.

At FWS-CWs, as the wastewater flows through the system, it is treated by a set of physical, chemical and biological processes. Vegetation promotes sedimentation by reducing water column mixing and resuspension of particles from the sediment surface, while attached and suspended microbial population degrade aerobically as well as anaerobically soluble organic compounds (Kadlec & Wallace, 2009). In fact, aerated zones occurs near the water surface due the re-aeration by atmospheric diffusion, that is the major oxygen source in this type of wetland, while anoxic and anaerobic zones prevail in and near the deeper zones due to the absence of oxygen and light (Kadlec, 2002). However, in heavily loaded FWS-CWs, the anoxic zone can move quite close to the water surface (Kadlec & Wallace, 2009; Vymazal, 2010).

Nitrogen is normally removed by the combination of nitrification/denitrification processes and by ammonia volatilization (Vymazal, 2005). Nitrification occurs in water column and following denitrification in anaerobic deeper zones (Crites, 1994). Regarding to phosphorus removal, these systems are not very effective because the major process in phosphorus removal in wetland systems (precipitation with ions of aluminium (Al), iron (Fe) and calcium (Ca) and/or adsorption to the medium surface particles) is limited by the little contact between wastewater column and the media substrate (Vymazal *et al.*, 1998; Taylor *et al.,* 2011). The plants can also contribute to treatment through uptake nutrients and other wastewater constitutes, but this function only represents a temporal storage because these elements are later released to water after the plant decay (Vymazal, 2005; Kadlec & Wallace, 2009).

An advantage of FWS-CWs is that mimics natural wetlands and hence they tend to bring back natural habitats, attracting various species of wildlife such as amphibians, birds and mammals and so, promote biodiversity of the ecosystems. The major disadvantages of these systems are that they generally required a much larger land areas than the subsurface flow systems and operated in a restricted temperature range because of the sensitivity of the ecosystem (Vymazal, 2010).

In fact, as we mentioned earlier, FWS-CWs are not favoured type in cold climates because they tend to freeze over the winter season, which results in significantly lower contaminant removal rates. These lower removal efficiencies are also associated with lower losses by volatilization and lower oxygen transfer rates (Kadlec & Wallace, 2009). These systems can also have problems related to the development of mosquito's populations and release of odours, especially when subjected to high organic loads, which can be harmful to public health, in addition to hinder people's acceptance (Tilley *et al.*, 2008). So, FWS-CWs are generally used to treat low to moderately polluted water despite operation costs for this system being the lowest of these types of treatment systems (Kadlec & Wallace, 2009).

2.2.2. Subsurface flow systems (SSF-CWs)

In Europe, until 2000, over 95% of the CWs were SSF systems (Platzer, 2000). The same author also refer that it is to be expected that, in Europe, the total number of these type systems exceeds 10.000 plants, of which more than 90% will be in the size range of 4 to 10 p.e. Also most of CWs in the U.S.A and elsewhere in the world are of this type and they are mainly used for tertiary treatment, promoting the removal of low concentrations of nutrients (N and P) and remaining suspend solids, while in European countries, such as Portugal, they are usually used to provide secondary treatment of municipal wastewater (Albuquerque *et al*., 2009a; Vymazal, 2010).

In Europe, these systems are generally coupled to septic and Imhoff tanks where the inffluent undergoes a pre-treatment in order to remove part of the suspended solids and oil and grease, aiming to prevent clogging of media and clogging of pipes. SSF-CWs have as main characteristic the fact of the water level remains below the top of the substrate so that the free water is not visible.

SSF-CWs also consist of channels or basins with impermeable bottoms with either clay or a synthetic membrane to prevent seepage to the groundwater. They are filled with granular media such as soil, sand, gravel media (or a mixture of these materials) or other less conventional materials (*e.g.* slag, shale, zeolites) to support emergent vegetation and to provide high void space to enable wastewater quickly infiltrate through the bed. Emergent vegetation is planted at the surface of the wetland bed and their roots extend into the saturated bed. The water surface is maintained at an elevation just below the surface of the bed. The bottom slope, porosity of the medium and daily average flows are critical design factors that must be considered to maintain the proper hydraulic gradient of the wastewater as it passes through the bed (Kadlec & Wallace, 2009).

Unlike FWS-CWs, in these wetlands the outlet structure is placed below the level of the inlet, forcing the wastewater to flow through porous substrate media before it exits (Brix, 1994; Kadlec & Knight, 1996) and the treatment occur during the contact with the surface areas of the medium particles and the roots of the plants (Morel & Diener, 2006). So, in contrast to the FWS-CWs, the substrate media contributes to the treatment processes by providing surfaces area for bacteria to attache, for chemical mechanisms of adsorption and is also responsible for physical filtration process, which results in a lower area demand and generally higher treatment performance per unit of area than FWS-CWs (Wallace & Knight, 2006; Vymazal, 2010). The major disadvantage of these wetlands is the clogging of the pores caused by the potential accumulation of suspended solids present in wastewater.

These systems are usually classified according to the hydraulic flow directions as either horizontal flow (**HSSF-CWs**) or vertical flow systems (**VSSF-CWs**) (Figure 2.1). In a typical horizontal flow system, wastewater is maintained at a constant depth and flows horizontally below the surface of the granular medium (Vymazal, 2005), while a vertical flow systems can be operated in downflow (percolating) or upflow mode, with the former technology being widespread in the treatment of domestic wastewater (Brix & Arias, 2005). Vertical upflow wetland systems are an emerging type of CW that has not been discussed much in the literature, in which the flow is applied continuously and have been used for treat wastewater containing volatile substances like as volatile organic compounds (VOCs) (Vymazal, 2010).

Vymazal (2005) points out that the vertical downflow system were originally developed by Seidel in 1967 and are shallow excavations or above-ground constructions that are generally characterized by intermittent loading and consequently resting periods where the wastewater is distributed over the surface of the wetland and gradually percolating vertically through the granular medium where coming in contact with the dense microbial populations on the surface of the media particles and is collected by drainage network at the base (Figure 2.4). Since the wastewater infiltrates through the filter bed, this type of wetlands are also called “infiltration wetlands” (Sundaravadivel & Vigneswaran, 2001).

In these systems, the water remain under the surface of the basin and usually they are planted with the same plants and the same densities (generally 4-8 plants.m^{-2}) that are used as for the HSSF systems and the main purpose of plant presence seems to be to help maintain the hydraulic conductivity of the bed (Vymazal, 2010). Different types of filling medium have been used in these systems which are made up of several layers of various grades of gravel and sand, from a layer of sand at the surface through to a layer of stones laid over the drainage system at the bottom as shown in Figure 2.4, with a typical depth of 0.5-1.2 m and a slope of 1% (Sundaravadivel & Vigneswaran, 2001; Kadlec & Wallace, 2009).

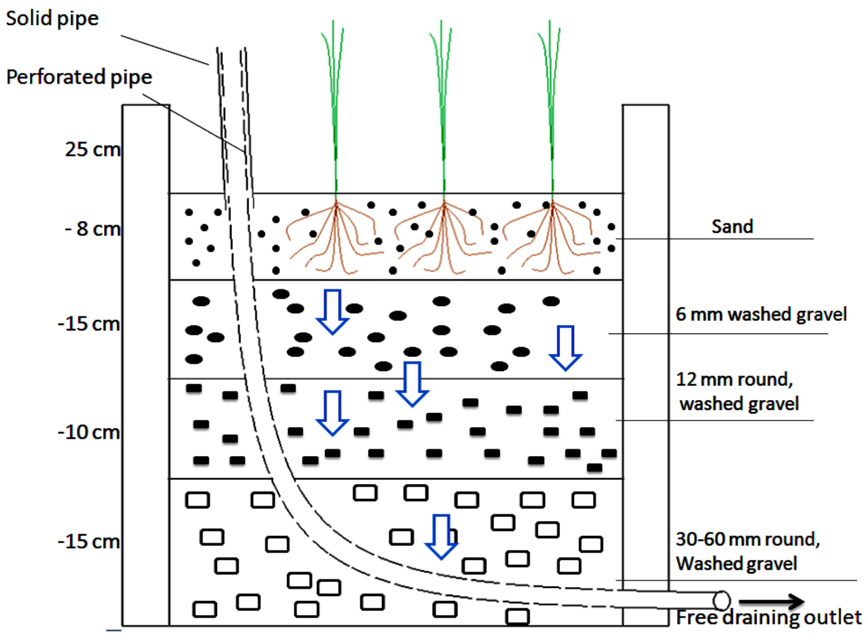

Figure 2.4 Schematic cross-section of a downflow VSSF-CWs system (Vymazal *et al.* 1998)

VSSF-CWs are primarily used to treat domestic or municipal sewage, however, they have also been successfully applied to industrial (food processing, acid mine drainage, among others) as well as agricultural (aquaculture, swine feedlot) wastewaters and in most cases has been also often used as part of hybrid systems treating various types of wastewater (IWA, 2000; Vymazal, 2011). Besides eventually clogging can occur, the VSSF wetlands require pumping of wastewater in order to introduce water to the system, although area demands are lower than for HSSF systems (Kadlec & Wallace, 2009). According the same authors, to reduce the risk of clogging, it is important to do an adequate primary treatment as pre-treatment and an OLR preferably lower than 60 kg BOD_5.ha^{-1}.day^{-1} (6g BOD_5.m^{-2}.day^{-1}).

Today, VSSF systems are becoming more popular and the reason for the growing interest in using these systems is related with the fact they have much greater oxygen transfer capacity than HSSF, resulting in better nitrification conditions. In fact, the usually use of intermittent loading seems to enhance oxygen transfer and thereby improved the capacity for decompose the organic matter and to nitrify ammonia nitrogen (Vymazal, 2009). Platzer (1998) cited by Vymazal (2005) referred that the intermittent loading system has a potential oxygen transfer of 23 to 64 $g.O_2.m^{-2}.day^{-1}$. Another advantage of this system is related with the need of smaller area for secondary domestic wastewater treatment (2-3 m^2/p.e.) than HSSF system which need 3-5 m^2/p.e. (USEPA, 2000).

In the other type of SSF systems, **HSSF-CWs**, the substrate media is maintained water-saturated through continuous application of wastewater (Vymazal, 2005). The effluent is fed at the inlet through a flow distribution tube placed under the bed surface and across its entire width and flows slowly and horizontally through the porous medium under the surface of the bed until it reaches the outlet zone where it is collected before leaving via level control arrangement at the outlet (Figure 2.5) (Wallace & Knight, 2006). The inlet is at one end and the outlet is at the opposite end and wastewater is maintained at a constant depth. According Vymazal & Kröpfelová (2008), today the most commonly medium used is washed gravel with grain size of about 10-20 mm and the bed depth is typically between 60 and 80 cm. The bottom of the bed is sloped (1%) to minimize flow above the surface.

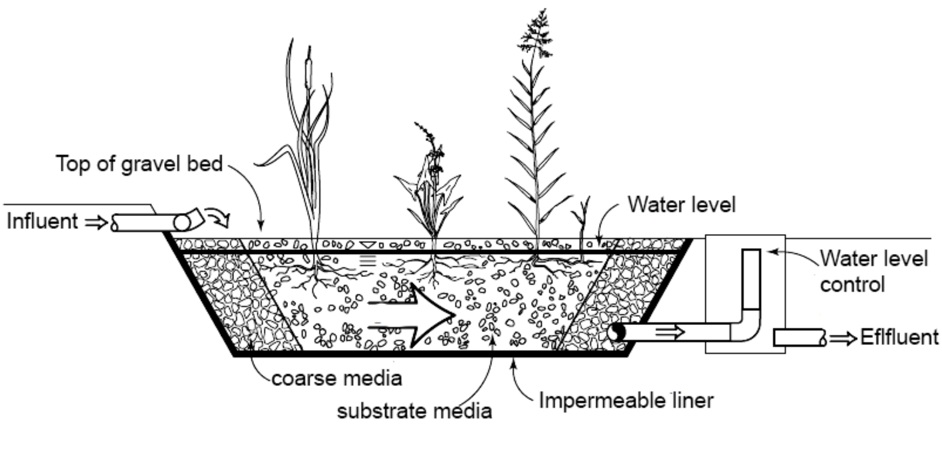

Figure 2.5 Schematic cross-section of a Horizontal subsurface water flow constructed wetland system (Wallace & Knight, 2006)

The first full-scale HSSF system was built in 1974 in Othfresen, Germany (Vymazal, 2005), and the early systems built in Germany and Denmark used predominantly heavy soils, often with high content of clay, which has resulted in serious problems of clogging of these systems, despite they have shown good treatment performance, due to its low hydraulic permeability. These systems are technically simple and economically attractive due to their low maintenance costs. If a slope can be employed, no pumping is necessary, which determines low energy consumption; however, bed volume utilization tends to be suboptimal, causing higher area demands than VSSF (Kadlec & Wallace, 2009). The most common wetland plant used in these CWs is common reed (*Phragmites australis*), nevertheless reed canary-grass (*Phalaris arundinacea*), sweet mannagrass (*Glyceria maxima*) and cattails (*Typha* spp.) are also frequently used (Vymazal, 2010).

In this type of CWs the bacteria attached to plants underground organs (roots and rhizomes) and media surfaces, contribute to degrade both organic compounds aerobically as well as anaerobically (Faulwetter *et al.*, 2009). However, some research studies have shown that the oxygen available in this type of CWs can be insufficient to ensure aerobic decomposition and so, anoxic and anaerobic decomposition may be more important (Vymazal, 2010; Chavan & Dhulap, 2012). Total suspended solid (TSS) that is not removed in pre-treatment systems is removed by filtration and sedimentation within the system and appears to take place primarily in the inlet zone of the bed (IWA, 2000).

CWs with HSSF seem to require much larger planted bed area in order to effectively eliminate phosphorous (P) and nitrogen (N) than the VSSF systems (Brix, 1994). As we will see in section 2.4.3 the N can be removed in HSSF-CWs by multiple mechanisms, although in this type of wetland system the primary mechanism for removal seems to be via the nitrification followed by denitrification (Zhang *et al.*, 2011; Wu *et al.*, 2014). Once the HSSF-CWs tend to have oxygen concentrations that seem to be insufficient to ensure complete nitrification, it can be expected that such systems tend to have lower removal rates of nitrogen than those presented by VSSF systems.

Phosphorus is removed from wastewater in HSSF wetlands primarily by adsorption exchange reactions, in which phosphate displaces water or hydroxyl ions from the surface of aluminium and iron hydrous oxides or precipitates as calcium phosphate, but generally the removal of phosphorus in HSSF also tends to be relatively low, unless that use materials with high capacity of adsorption in the filling medium (Cui *et al.,* 2008; Mancilla Villalobos *et al.,* 2013).

The main operational problem of this type of CWs is the potential problem related with the progressive clogging of the porous media, which contributes to diminishes the hydraulic performance and consequently can be affect the pollutant removal efficiency and lifetime of the system (Albuquerque *et al,* 2009b; Knowles *et al*., 2011; Chavan & Dhulap, 2012). Also, at the last decade we have witnessed attempts to technological adaptations in order to improving the efficiency of pollutant removal on this type of CWs systems, such as forced-bed aeration and baffled flow CWs (Sayadi *et al*, 2012; Butterworth, 2014; Wu *et al.,* 2014). However, these improvements usually result in an increased in operational and energy costs compared with conventional constructed wetland systems.

2.2.3. Hybrid systems

Different types of CWs may be combined with each other (so called hybrid or combined systems) in order to utilize the specific advantages of the different types and to achieve higher treatment effectiveness, especially for nitrogen removal (Vymazal & Kröpfelová, 2008; Avila *et al*., 2014). Although there are other types of combinations, hybrid systems are comprised most frequently of a first stage with a VSSF used to remove organic and suspended solids and to provide nitrification followed by a HSSF for denitrification (Figure 2.6), and usually these systems include either single-cell or combined multiple-cell systems (in series or in parallel) (Kadlec & Wallace, 2009; Vymazal, 2013; Gikas & Tsihrintzis, 2014).

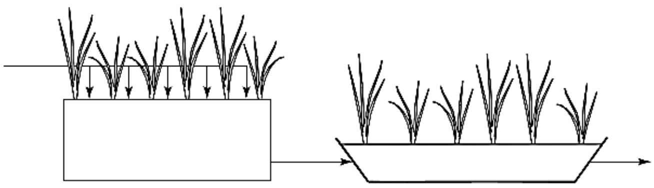

Figure 2.6 Schemes of the most common hybrid wetland systems (Kadlec & Wallace, 2009)

The results reported in literature indicate that generally combined systems provide better efficiencies than individual systems, with very good removal for organics (BOD_5 and COD) and total suspended solids (TSS) (Vymazal, 2013). Also for nitrogen removal, these systems seem to be more effective with nitrification taking place in vertical flow stage and denitrification occurring at HSSF, with a final effluent which is fully nitrified and partly denitrified and hence has much lower TN outflow concentrations (Cooper *et al.,* 2010; Sayadi *et al*., 2012). Therefore, in late 1990s, multi-state systems were used with increasing frequency in order to provide higher breakdown of pollutants presenting in wastewaters, especially nitrogen removal due the potential inability to produce simultaneously nitrification and denitrification in a single horizontal or vertical flow, and thereby contribute to optimize the performance of these natural treatment systems.

Generally, the main advantages of CWs over conventional wastewater treatment technologies include the following: lower operational costs, less energy demand and they produce small quantities of by-products which do not need further treatment (Rai *et al*., 2013; Gikas & Tsihrintzis, 2014; Wu *et al*., 2014). However, the lifetime and wastewater treatment efficiency vary widely and one significant disadvantage of these systems is their higher area requirement, despite, this issue makes CWs better adapted to changing hydraulic and nutrient loadings, and therefore more appropriate for small settlements, which are characterized by variable wastewater flow rates and pollution loads (Albuquerque *et al.*, 2009a). The Table 2.1 shows the advantages and disadvantages of each system type of CW.

Table 2.1 Advantages and disadvantages of three main types of CWs (Adapted from Tilley *et al.,* 2008; Kadlec & Wallace, 2009; Vymazal, 2011; Knowles *et al*., 2011; Gikas & Tsihrintzis, 2014)

Type of CWs	Advantages	Disadvantages
FWS	• Easy to construct with locally available materials; • Moderate to low operation and maintenance costs and no energy needed except for pumping in case of flat topography; • Resistance to shock loading and minimal sludge production and excellent landscape integration; • Removal efficiencies are dependent of climate and type of wastewater, but generally following ranges may be expected: 90% BOD_5, 60-90% TSS; 20% TN; 20% TP and 95-99% of pathogen removal.	• Large areas are required which can mean high capital costs; • Relatively long start-up period to work at full capacity; • Seasonally dependent removal; • Potentially may facilitate the risk of mosquito, rodent proliferation and odour nuisance; • Can be an area for transmitting diseases due to the potential exposure of human and animals to wastewater.
VSSF	• Easy to construct with locally available materials; • Moderate construction, operation and maintenance costs and no energy needed except for pumping in case of flat topography; • Resistance to shock loading and low sludge production; • Less odour nuisance and mosquito proliferation than FWS-CWs because of belowground water; • Removal efficiencies are dependent of climate and type of wastewater, but generally following ranges may be expected: 90-95% BOD5, 80-90% COD; 90-95% TSS; 50-70% TN; 20-30% TP (usually tend to decline with time) and 99% of pathogen removal.	• Seasonally dependent removal; • Relatively long start-up period. • To prevent clogging requires the use of mechanically pre-treated wastewater (The distributing wastewater system requires more complex engineering and maintenance when compared with FWS).
HSSF	• Easy to construct with locally available materials; • Moderate construction, operation and maintenance costs and no energy needed except for pumping in case of flat topography; • Resistance to shock loading and low sludge production and easy integration in landscape; • Less odour nuisance and mosquito proliferation than FWS-CWs because of belowground water; • Removal efficiencies are dependent of climate and type of wastewater, but generally following ranges may be expected: 85-95% BOD5, 80-90% COD; 90-95% TSS; 20-50% TN; 20-30% TP and 99% of pathogen removal.	• Relatively high area requirement; • Relatively long start-up period. • Seasonally dependent removal; • To prevent clogging requires the use of mechanically pre-treated wastewater (The distributing wastewater system requires more complex engineering and maintenance when compared with FWS); • Denitrification are effective, but may have problems to ensure complete nitrification due to the limited oxygen transfer.

2.3. Main Components of HSSF-CWs and its functions

HSSF is the treatment wetland technology mainly chosen, in the last two decades, by *AdP*-Águas of Portugal S.A., to provide secondary municipal wastewater treatment to small agglomerations (i.e. populations with less than 2000 inhabitants). In fact, according to the Classification System of Fonder & Headly (2010) (Figure 2.1), the system that prevails in Portugal is HSSF-CW Type 4: horizontal subsurface flow system with emergent non-woody vegetation. In Portugal, the configuration of these systems usually consists of a relatively shallow rectangular basin (usually with a 0.3 to 0.6 m deep), which is planted with the common reed (*Phragmites australis*) and/or other emergent macrophytes species. For preventing a build-up of solids in the inlet area, odour nuisances, clogging of the bed or blockages of the distribution pipes, in Portugal, the pre-treatment is normally setting up in septic tanks or Imhoff tanks.

The growing popularity of HSSF-CWs can be attributed to technical advantages and ecological benefits (Table 2.2) that have contributed for a positive public perception and acceptability of this technology and to be recognized as a sustainable eco-technology, especially useful for treatment of domestic wastewater. Due to their high buffering capacity these systems show a very robust treatment performance and usually presenting a relatively stable quality of effluent (Langergraber *2013*; Galvão & Matos, 2012).

Table 2.2 Major technological and ecological benefits linked to HSSF-CWs (Adapted from Matos *et al*., 2009; Knowles *et al*., 2011; Wu *et al.,* 2014)

Technological Benefits	Ecological Benefits
Can be less expensive to build than other conventional treatment alternatives.	Great landscaping and ecological integration, with low environmental impact associated with these infrastructures.
Operation and maintenance costs (energy and chemicals) are low compared to conventional systems.	They seem to contribute for habitat conservation and biodiversity for plant, bird, animal, insect and others aquatic life.
Operation and maintenance requires little Know-how and periodic on-site labour, i.e. unlike conventional systems do not require full time supervision.	They can be built to fitting harmoniously into the Landscape.
As other CWs systems, they are able to tolerate fluctuations in flow and pollutant loading, which makes resilient in the treatment of a wide range of waste water and in the most varied environmental conditions.	Value added crop cultivation, such as ornamental plants and plant biomass can be harvested for use as an energy crop.
They can treat a wide range of pollutants, often simultaneously.	Aesthetic enhancement of open spaces.
They facilitate water reuse and thus can contribute to the conservation of water resources.	Social benefits such as education and recreation (nature watching, exercise, hunting). They are an environmentally sensitive approach that is viewed with favour by the general public.

HSSF-CWs consist of four main basic components: substrate media, vegetation, microbial communities and water and their efficiency in removing pollutants seems to depend on the root zone interactions between substrate media, pollutants, plant roots and a diversity of microorganisms. The understanding of the different components and its functions is essential for the operation, management and potentially for the handled of HSSF-CWs.

2.3.1. Substrate media

Substrates media carry out many different purposes in HSSF-CWs and play an important role in physical, chemical and biological processes that are responsible for pollutants removal from wastewater (Cui *et al.,* 2008; Meng *et al*., 2014). They are an essential component in supporting the growth of emergent plants by allowing penetration of root system and as a nutrient source for its establishment. The substrate media also provides a stable surface area for microbial growth to occur which are the main responsible for biological treatment of wastewater within HSSF-CWs. This component is also important to the distribution/collection of flow at inlet/outlet, and has an important role in filtration, sedimentation and adsorption of pollutants (IWA, 2000).

Materials with smaller diameter (e.g. sand) can be more effective in removing pollutants through physical processes due to the formation of smaller pores sizes and in adsorption removal mechanisms because they provide larger surface area than materials with higher diameter (e.g. gravel) (Cui *et al.*, 2008; Albuquerque *et al.*, 2009b). However, an ideal filtration media must provide not only a high filtration effect, but must also guarantee a satisfactory hydraulic conductivity (Kadlec & Wallace, 2009). In fact, the substrate media, especially its grain-size distribution, can strongly influenced the movement of water through the wetland bed and consequently the HRT which affect the contact time that is necessary between the wastewater and the biofilm for an adequate biodegradation (Albuquerque *et al*., 2009b; Vymazal, 2010). Very small particles have very low hydraulic conductivity, whereas large particles have high conductivity and low surface area per unit volume suitable for attached microbial communities.

Several materials could be used as filter material in CWs, depending on the selection of the type and size of materials, on the characteristics of pollutants and its concentration, the desired outcome and also of the hydraulic regime chosen (García *et al*., 2010). It’s also important to take into account the economic viewpoint and, therefore, choise should be made on locally available materials in order to reduce costs.

Originally, to reduce the cost of construction the use of soil that had been extracted from the site during the bed construction was proposed as a substrate media. However, it was observed that the suspended solids presented in the wastewater rapidly clogged the pore spaces of this media, which consequently contributes to reducing hydraulic conductivity of the bed. It was also found that the plant roots did not contribute to increase or stabilize the hydraulic conductivity of the soil as it was expected (Brix, 1997; Vymazal, 2005). So, the use of larger rock sizes (10-15 cm) that ensured larger void spaces and that would also offer less resistance to flow was proposed, but plants that usually grow in soils also have difficulty in developing its root system in a substrate media that has large void spaces and as already mentioned, the larger rocks provided a smaller surface area for the support of microbial growth compared to the smaller sizes.

Considering all these factors, an intermediate-sized material started being used. In some countries, such as Portugal, the most widespread systems include the use of combinations of successive layers of different diameter such as gravels, sand and also one layer of top soil in order to achieve maximum performance. The main purpose of the top soil layer with small diameter material seems to provide better root establishment and properly develop bed root system. The mixture of sand and gravel also seems to produce best results in terms of both hydraulic conditions and the pollutants removal (Stottmeister *et al.*, 2003; Albuquerque *et al.*, 2009b).

At the inlet and outlet zones it is recommended that the media should be larger in diameter to minimize clogging and should increase from the top to the bottom of the system (Davison *et al.*, 2005). The diameter recommended to these zones varied with authors: USEPA (2000) recommended washed gravel with a diameter between 40 and 80 mm, while Cooper *et al.* (1996) considered a material ranging from 50 to 200 mm of diameter. In Portugal, gravel with a diameter of 80-150 mm is typically used, which has good hydraulic conductivity and ensure an adequate distribution of the affluent (Galvão, 2009).

Regarding to the effective treatment zone, typical sizes of the media vary between 2 and 128 mm, although it does not appear to be any advantage in terms of pollutant removal of using substrates whose diameter comes out of range values between 10 and 60 mm (USEPA, 2000). In Portugal, it is common to use in the treatment zone a configuration that includes a layer of gravel (15/25 mm) at the base with a thickness of about 20 cm, then a layer of 30 to 40 cm of coarse sand (3/10 mm) and finally a topsoil layer whose thickness is about 10 cm is placed on top of the bed. The maximum bed depth should be 1.0 m in the downstream section (Albuqerque et al., 2009b; Galvão, 2009). The characteristics of typical media used in HSSF-CWs are shown in Table 2.3.

Several authors have shown that the HSSF-CWs based on those traditional media are able of meet the required TSS, BOD_5 and COD reductions; however, with those materials, it is often difficult to reach significant removal of certain inorganic nutrients, such as orthophosphate and ammoniacal-nitrogen (Zhao *et al.,* 2008).

Table 2.3 Characteristics of typical substrate media for HSSF-CWs (USEPA, 2000)

Type	Effective grain size, D_{10}* (mm)	Porosity (%)	Hydraulic Conductivity ($m^3.m^{-2}.day^{-1}$)
Coarse sand	2	32	1000
Gravelly sand	8	35	5000
Fine gravel	16	38	7500
Medium gravel	32	40	10000
Coarse rock	128	45	100000

*D_{10} is termed as the effective particle size and means that 10 percent of the particles are finer and 90 percent of the particles are coarser than that particular particle size D_{10}.

Therefore, presently others materials have started to be under investigation as alternative for bed media such as industrial by-products or waste materials from industries like alum sludge (waste from drinking water treatment plant), slag (by-product from the steel making industry), cement clinker (from cement industry), artificial products like light-weight expanded clay aggregates (LECA) among others, as well as natural materials with potential higher adsorption capacities (e.g. calcite, dolomite, shale, peat, limestone and natural zeolites) in order not only to minimize the clogging problem, but especially to enhance the performance of HSSF-CWs since they present both higher porosity and specific surface area, which allow a better biofilm adhesion and adsorption capacity (Zhao *et al.*, 2008; Albuquerque *et al.*, 2009b; Babatunde *et al*., 2010; Tao & Wang, 2009).

Zhao *et al.* (2008) have been exploring the use of dewatered alum sludge as substrate media in two laboratory scale HSSF reed beds, using farmyard wastewater as the influent and they have observed a removal efficiency of 78% for BOD_5, 82% for COD and 93 % for TSS under a hydraulic loading of 0.80 $m^3.m^{-2}.day^{-1}$. They also have not observed any serious problems of operation such as clogging. Regarding to phosphorus removal rates, they also reported a good removal rate (92%) at a loading of 21.5 g PO_4^{3-}-$P.m^{-2}.day^{-1}$ concerning a long time operation of 193 days. However, those authors considered that is important not only to confirm these results in systems on larger scale, but especially evaluate the aluminium release along the time.

Drizo *et al.* (2006) study the physic-chemical properties of 57 different potential substrate media and found that slag, a compound of silica with aluminium oxides and oxides of calcium and magnesium, had the highest phosphorus retention capacity, while Dallas & Ho (2005) have been conducted an experiment using plastic bottle segments as an alternative low cost substrate for treatment of domestic wastewater in Monteverde, Costa Rica and they observed that BOD_5 and faecal Coliforms removal was better than that of crushed rock systems. The use of natural zeolites (volcanic materials that are hydrated alumina-silicates of alkali with crystalline structure) in environmental applications is also increasing due to their net negative charge and so cation exchange ability, very large surface area and significant worldwide occurrence. Also the LECA material has shown both good water permeability and phosphorus removal capability by adsorption (Van Deun & Van Dyck, 2008; Albuquerque *et al*., 2009b; Meng *et al.*, 2014).

The major concern with bed depth is the ability of plants to extend their roots throughout the bed depth in order to provide oxygen for aerobic degradation of organic matter and oxidation pf ammonium nitrogen. Typical HSSF-CWs has a filtration bed depth of 0.2-0.8 m in order to allow roots of wetland plants to penetrate the whole bed and so have an effect on treatment, especially to ensure a best oxygenation of whole bed by oxygen release from root system, although it is generally recommended a depth of 0.6 m (Wallace & Knight, 2006).

2.3.2. Vegetation

The plants most used in HSSF-CWs systems are known as emergent macrophytes, that are herbaceous (soft tissue and non-woody) vascular plants (higher plants) and have a structure consisting of aerial stems, leaves and an extensive root and rhizome system. Their roots and rhizomes are under the substrate media, while the major portions of these plants emerge above the surface media and so are exposed to the air. This wetland species can grow within a water level ranging from 0.5 m below the soil surface to a water depth about of 1.5 m and they have different inundation tolerances depending on the species and depth penetration of the root system (Brix, 1994).

Generally, emergent macrophytes are perennial species which means that the plant lives and continuous for at least three growing seasons due to its belowground portions (roots and rhizomes) that remain dormant throughout the winter season and sprout again in the next growing season. The aboveground portion (stems and leaves) can either remain standing through the dormant season until the beginning of the next growing season (persistent) or die back to the ground and decay (non-persistent). Moreover, wetland plants show different biomass production rates (growth rates) and prefer specific environmental conditions (e.g. temperature, light demand, salinity tolerance and pH) (Brix, 1994; Vymazal *et al*., 1998).

Plants are major component of wetlands but several studies on their effects in wastewater treatment have revealed contradictory results. Some authors reported that plants have little influence on wastewater treatment efficiency (Kadlec & Knight, 1996; Vacca *et al.*, 2005), while others studies emphasized that their presence in HSSF-CWs are crucial and some researchers noted significant effects of plants with an increased in final effluent quality and higher nutrients removal rates (Picard *et al*., 2005; Yang *et al*., 2007; Akratos *et al.*, 2008). However, these contradictions probably often reflect the different plants selected or the season in which studies have been conducted.

Vegetation have many functions in HSSF-CWs that ensures that several processes involved in removing pollutants are carried out by either direct or indirect effects. As indirect effects of plants, we can referred the effect on the hydrology of wetland system by creating barriers to flow due to the root structure and growth, hence causing an increase in dispersion, a decrease in water velocity flux and so, promoting the sedimentation of suspended particles (Stottmeister *et al.*, 2003; Vymazal, 2011). In addition, rhizomes and roots of macrophytes when well developed in the substrate media also participate in stabilizing the bed against disturbances that may come from water flux or environmental conditions and also reduce the potential resuspension of solids particles (Brix, 1994).

Another important aspect of plant's role is their effect on water balance in HSSF-CWs. Wetlands receives water through wastewater influx and potential rainfall, and loses water to outflow and evaporation. So, plants have a key role in dynamics of water loss, essentially by controlling these losses through evaporation and transpiration, i.e. evapotranspiration (ET) (Chazarenc *et al*., 2010; Galvão *et al.*, 2010; Bialowiec *et al*., 2014). Through ET, the plants contribute for reduce the volume of water within the CWs and so to decrease the outflow and, at the same time, also contribute to increase the HRT in summer time in temperate regions, such as the case in Portugal, which allows more interaction time between wastewater and wetland ecosystem.

According to El Hamouri *et al.* (2007) the ET can be seven to eight times higher than evaporation in CWs without plants. The same authors reported an ET in Morocco planted with *Arundo donax* was 40 mm day^{-1} and nearly 60 mm day^{-1} with *Phragmites australi*s, as compared to 7 mm day^{-1} in an unplanted HSSF-CW. Borin *et al.* (2011) found a similar water loss from a system also planted with *Phragmites australi*s in a relatively humid zone in Italy. Also, in a flow simulation study in HSSF-CWs operating in Portugal, Galvão & Matos (2012) observed a significant influence of that variable on daily flow reductions and therefore they concluded that the evapotranspiration rates can be relevant thus influencing the pollutant mass discharged, especially during summer months. In fact, as ET reducing the volume of wastewater can also contribute for concentrating the pollutants in outflow, which in some regions where treated wastewater may have as destine the reuse in agricultural soils, may be disadvantageous (Marecos do Monte & Albuquerque, 2010; Headley *et al.*, 2012).

The root system of plants also provides additional sites for biofilms development and it is generally assumed that planted wetlands outperform unplanted controls mainly because active microzone around the root of wetland vegetation, also called rhizosphere, that seems to stimulate the microbial density, diversity and activity by providing not only root surface for microbial attached, but also a source of carbon compounds for some heterotrophic microorganisms through root exudates and a micro-aerobic environment via root oxygen release favourable to some aerobic microorganisms (Münch *et al*., 2005; Gagnon *et al.,* 2007; Faulwetter *et al*., 2009). However, Costa *et al*. (2013) studying HSSF-CWs with 4 years of operation (which according to Brisson & Chazarenc (2009) is a period considered sufficient for the complete plant development) observed similar treatment efficiencies for planted and unplanted systems, having Vacca *et al.* (2005) achieved similar results.

The plants can also directly be involved in the treatment processes by absorbing some pollutants presented in wastewater that are essential to its plant biomasss growth, such as nitrogen, phosphorus which are incorporated into the plant structure. However, the contribution of vegetation in nutrient removal is controversial. According to Brix (1997) and Brisson & Chazarenc (2009), the direct uptake by plants depends on plant species used and characteristics of influent, particularly nutrient loading rates and its impact in removing pollutants may only be significant when wastewater nutrient levels are low. Therefore the results reported in the literature regarding the role of uptake of nutrients by plants in terms of their total removal in these systems is highly variable (ranging from 3% to 66% for nitrogen and 3% to 60% for phosphorous) (Langergraber, 2005; Shelef *et al.,* 2013).

The concentration of oxygen within substrate media is of critical importance in HSSF systems because these wetlands are permanently saturated and in such conditions water replaces the atmospheric gases in the pore spaces and can affect the aerobic respiration plant's roots and its nutrient availability, as well as influences microbial respiration and chemical oxidation processes (Armstrong & Armstrong, 1990; Brix, 1997; Stottmeister *et al*., 2003).

In a waterlogged media, the pore spaces are filled with water and the rate of oxygen diffusion through water and substrate media is slow, about 10^4-10^6 times slower as it is through air, mainly due to the smaller diffusion coefficient in water and low solubility of oxygen in water (Vymazal, 2011). So, the macrophytes have developed anatomical and morphological adaptations that allow the internal transport of oxygen from their aerial organs to their root system. This important adaptive mechanism compared to terrestrial plants is the presence of the aerenchyma or aerenchymous tissues: an aerating tissue containing large intercellular gas spaces (lacunae) that form a hollow connecting channel system from the leaves and shoots to the roots and rhizomes, and vice versa (Armstrong & Armstrong, 1990; Brix, 1994; Sorrell & Armstrong, 1994).

Some studies reported that part of the oxygen which arrives at the extensive root system of wetland plants (Figure 2.7a) may diffuse from them to adjacent layer, creating oxidized microzones around the roots surface (Figure 2.7b) that could contribute for supporting more efficient aerobic treatment removal of organic and nitrogen compounds in HSSF-CWs (Brix, 1997; Ruiz-Rueda *et al*., 2009). According to Armstrong & Armstrong (1990), this oxygen may represent more than 90% of the total oxygen entering in HSSF-CWs systems.

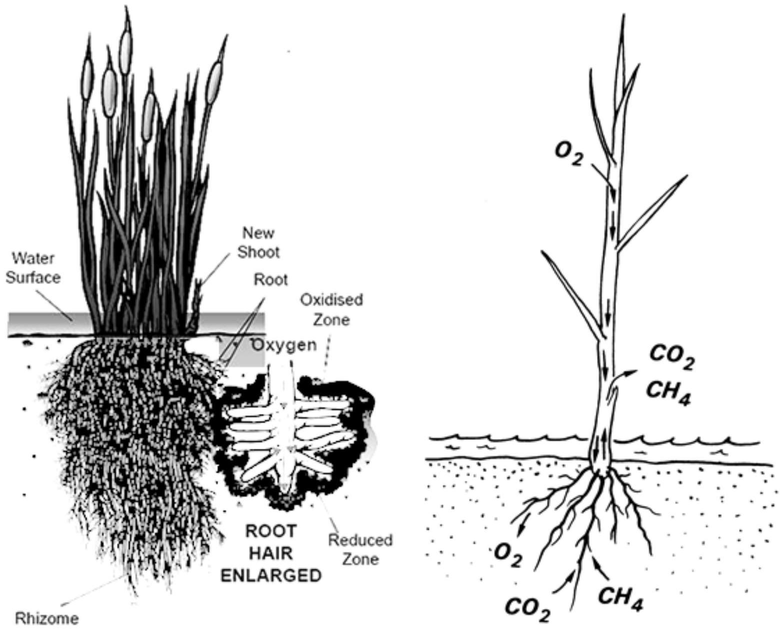

Figure 2.7 The extensive root system of wetland plants (a) and oxygen releases within the rhizosphere (b) (Dubey & Sahu, 2014 (a); Brix, 1994 (b))

The rate of oxygen release by roots of plants seems to depend on several other factors such as redox potentials and oxygen levels in the rhizosphere (Dong *et al.*, 2011) and of the plants species due to differences in vascular tissues, metabolism, and root distribution (Stottmeister *et al.,* 2003; Picard *et al*., 2005; Wiessner *et al.*, 2005). Sometimes, different oxygen release rates even for the same wetland plants are also referred. In fact, Brix & Headley (2007), observed poor oxygenation in rhizosphere of *Phragmites australis*, having reported an amount of oxygen leaked from the root system of 0.02 $gO_2.m^{-2}.day^{-1}$, which have been considered of no quantitative importance for aerobic organic matter degradation and microbial nitrification in the rhizosphere, while Armstrong & Armstrong (1990) reported for the same plant values about 5-12 $gO_2.m^{-2}.day^{-1}$. Sorrell & Armstrong (1994) observed a value for the oxygen release rate of 126 $\mu molO_2.hour^{-1}.gram^{-1}$ of dry weight (dw) of root for *Juncus ingens*, while Chabbi *et al*. (2000) found a value of 1.5 $\mu molO_2.hour^{-1}.gram^{-1}$ dw for same species. Reddy *et al*. (1990) reported a rate of oxygen release of 0.4 $mgO_2.day^{-1}.gram^{-1}$ root dw for *Typha latifolia*.

Dong *et al.* (2011) observed the oxygen release rate of wetland plants shows diurnal fluctuations due to differences in light intensity what they have considered the major factor influencing oxygen release rates. Seasonal cycles of plant growth may also influence oxygen release and so, consequently may determine seasonal different wetland performance. In fact, Taylor *et al*. (2011) in a study with different species has observed that wastewater treatment was affected by seasons and plant species. They was observed that many species had consistently high COD removal throughout the year, while others had intermediate or inconsistent seasonal COD removal, and, among other factors, attributed it to the difference in the capacity to release oxygen to the rhizosphere.

Therefore, in some cases the authors assume that the design of HSSF-CWs can be based in the principle that the amount of oxygen released from root system should be sufficient to meet the demand for aerobic degradation of organic substances presenting in the wastewater as well as for nitrification of the ammonium and agreed that plants can improve pollutants removal (Bezbaruah & Zhang, 2004; Karathanasis *et al*., 2003; Akratos & Tsihrintzis, 2007; Maltais-Landry *et al.*, 2009). However, others studies reported that oxygen release from roots of different plants is negligible and did not detected any significant difference between unplanted and planted systems (Baldizon *et al*., 2002; Vacca *et al.*, 2005). At present, most authors agree that the characteristics of HSSF-CWs promote anaerobic and anoxic conditions except for a few millimetres at the surface and at the rhizosphere (Vymazal, 2011; Chavan & Dhulap, 2012; Shelef *et al.*, 2013).

Other functions of plants in HSSF-CWs include aesthetic appearance by covering wetland bed, controlling potential odours and also may enhance wildlife values through providing an adequate habitat for many species. Usually, it is recommended a density of four to eight seedlings per square meter (m^2) to obtain a satisfactory coverage and to prevent freezing in the winter months, especially in cold and temperate regions (Picard *et al.*, 2005; Vymazal & Kröpfelová, 2008).

However, the presence of plants may also lead to some problems such as the accumulation of plant detritus that can contribute to internal nutrient loading and so their effects can be inverted and compromise wetland performance. It is also usually referred to as a negative effect of plants their potential contribution for enhance mosquito reproduction, while others authors referred that the aerenchyma tissue may play an important role in the emission of methane (CH_4) (greenhouse gas) (Figure 2.7), as well as in releasing nitrogen gas (N_2) and nitrous oxide (N_2O) (also a greenhouse gas) produced by denitrification of nitrate (NO_3) into the atmosphere (Søvik *et al.* 2006; Mander *et al*.,2014; Maltais-Landry *et al.* 2009).

Plant species are primarily selected according to their tolerance to local climatic conditions, but plants must be selected with high capacity for propagation, establishment and presenting a rapid and good growth. It is also important that plants have a large and well-developed root system (Figure 2.7a) because they both contribute to higher levels of biomass and probably ensures a better oxygenation of the substrate, which also contributes to a higher density and diversity of the microbial population (Brix, 1997; Vymazal, 2010).

A wide variety of wetland plants have shown these properties (Figure 2.8); however, bulrushes (*Sripus* spp.), cattails (*Typha* spp., especially *Typha latifolia)*, rushes (*Juncus* spp.) and reeds (*Pragmites* spp*.,* especially *Phragmites australis)* are the most commonly used plant species found in HSSF-CWs around the world and in many Europe countries, including Portugal. All of these plants have a large biomass above and belowground organs and the roots system grow horizontally and vertically, creating an extensive matrix which binds the substrate particles and ensure substrate for attached bacteria and oxygenation of areas adjacent to the roots and rhizomes (Vymazal, 2011; Shelef *et al.*, 2013).

Figure 2.8 Different types of plants growing in the HSSF wetland systems (Lavrova & Koumanova, 2013).

2.3.3. Microbial community

A suitable microbial community for wastewater treatment in HSSF-CWs develops spontaneously from the autochthonous microorganisms that were naturally and previously present in the substrate media and from the microorganisms that arrive with wastewater to be treated and may include bacteria, yeasts, fungi and protozoa (Samsó & Garcia, 2013; Arroyo *et al*., 2015). The plant roots system and media particles constitute the physical support for a large consortium of living microorganisms in HSSF wetland, which play an important role in pollutant removal, as well as in most conventional wastewater treatment plants, especially relating to labile forms of organic matter and to nitrogen transformations. Calheiros *et al.*, (2009), Samsó & García, (2013); Adrados *et al.*, (2014); Ansola *et al*., (2014); and Arroyo *et al*., (2015) published several studies on the microbial population that is present in CWs.

Adrados *et al.* (2014) in a study on the prokaryotic microbial communities of different systems operating in Denmark for treat domestic wastewater (HSSF-CWs, VSSF-CWs) have found that γ-Proteobacteria and Bacteroidetes are the most representative taxonomic groups and also found members of the Actinobacteria group. They also observed lower archaeal diversity in comparison with bacterial population. The same authors also observed in HSSF-CWs typical soil bacteria that are potential denitrifying bacteria such as *Acinetobacter* spp. (γ-Proteobacteria), *Arthrobacter* spp. (from Actinobacteria group, also found in samples from VSSF systems) and *Bacillus* spp. (Firmicutes, which were not present in VSSF).

The results obtained by Adrados *et al.* (2014) also showed the presence of aerobic microorganisms in HSSF systems, which suggest that although these systems are permanently saturated; there is sufficient oxygen to permit proliferation of these microbial groups and thus the possibility of occurrence of nitrification in the system. Similar results were obtained by Ansola *et al.* (2014) when have characterized the bacterial community structure of CWs.

Generally it was observed that microbial population structures in HSSF-CWs is spatially heterogeneous and tend to show different spatial distribution patterns along the length of the bed which is also reflected in a subsequent compartmentalization of biochemical processes (Figure 2.9) (Samsó & García, 2013). It was observed that microbial biomass and activity tend to decrease along the length of the bed and with depth due to changes in amount and composition of available substrates for the microorganisms along those profiles, as well as changes on some environmental conditions such as redox potentials, pH and temperature (Nurk *et al.,* 2005; Truu *et al.*, 2005; Samsó & García, 2013).

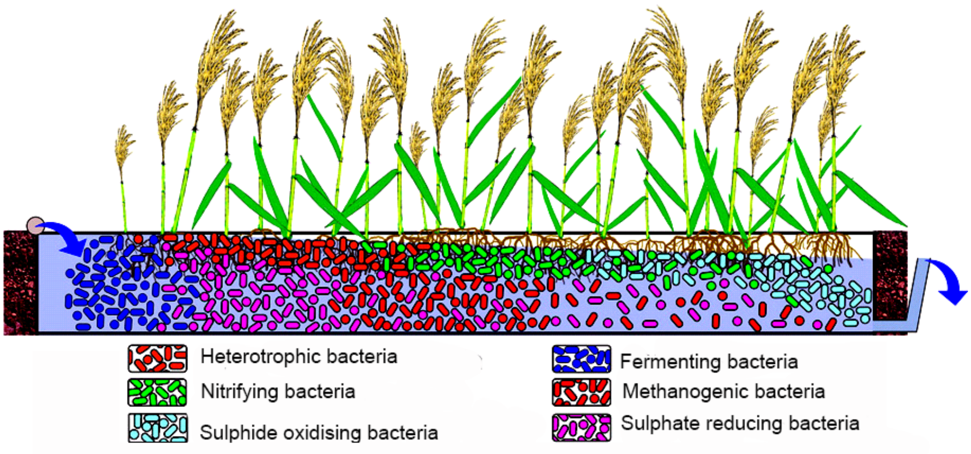

Figure 2.9 Schematic representation of spatial distribution of microbial population along of the longitudinal section of the pilot wetland (Samsó & García, 2013)

Several studies also reported that diversity and density of microbial communities in CWs seems to be related with the quantity and quality of organic matter load and nutrients (nitrogen and phosphorus) compounds concentrations presenting in wastewaters (Calheiros *et al*., 2009; Adrados *et al*., 2014). Arroyo *et al.* (2015) study the influence of environmental variables on the structure and composition of bacterial communities in CWs and they observed a clearly linked between bacterial structure and COD and nitrogen concentrations (NH_4^+-N and total Kjeldahl nitrogen (T_KN)), having found that, generally, the community composition is dominated by the phylum Proteobacteria. These results are similar to those obtained by Ahn & Peralta (2009) and Ligi *et al*. (2014). In the same study, Arroyo *et al.* (2015) also found that bacterial diversity were significantly lower in CWs which receives the highest concentrations and they also observed that seasonal factor was not relevant neither the composition nor the diversity.

Ahn *et al.* (2007) and Baptista *et al.* (2008) in HSSF-CWs observed that plants did not seems have significant effect on bacterial community structure, while Calheiros *et al.* (2009) found a positive relationship between plant species and the density and diversity of bacterial communities within rhizosphere of wetland plants. These results are consistent with those presented by other authors (Zhao *et al*., 2010; Arroyo *et al.,* 2015). Zhao *et al*. (2010) not only observed that planted wetlands enriched microbial communities when compared with unplanted systems, as well as found that occur differences in microbial community structure in the rhizosphere at different season and attributed these differences to the physiological and morphological alterations of plant during the vegetative cycle. Therefore, retention and proliferation of microbial communities may be intensified by manipulating plant communities and so, the benefits between plants and microorganisms can be often symbiotic and can have a positive effect on treatment effectiveness.

Generally, the bacteria seems to be preferentially attached to the solid material of the media in the rhizosphere and in lower numbers in the aqueous phase that fills the pores, whilst protozoa are present in the water that filled pores and at the water layer, which covers media particles and roots (Münch *et al.,* 2005). In the rhizosphere, root exudates and dead root matter can contribute for microbial growth as these compounds serve as substrates for them and, consequently in root zone it is expected that there is not only a high density, but also a great diversity of microbial community (Brix, 1994; Münch *et al.,* 2005).

The diverse bacterial enrichment is the driving force for wastewater treatment in both CWs and conventional sewage treatment plants. In fact, the microbial activity has a crucial role in transforming a great number of organic and inorganic substances into innocuous or insoluble substances because the organisms can use some contaminants for their own growth and reproduction. They feed on organic materials and/or nutrients thus reducing, breaking down or completely removing a wide variety of contaminants from the wastewater and they are also involved in the recycling of nutrients (Song *et al*., 2010; Meng *et al*., 2014).

These transformations can be aerobic or anaerobic, but based on the discussion earlier dealing with the limited availability of oxygen, it is expected that the most bacterial species in HSSF are facultative which means that they are able of functioning under both aerobic o anaerobic conditions in response to changing environmental conditions. The microbial community of a CW can be also affected by toxic substances, such as pesticides and heavy metals, and care must be taken to prevent such chemicals from being introduced at damaging concentrations (USEPA, 2000).

2.3.4. Water

The hydraulics and the internal flow patterns can be of great importance for the performance of HSSF-CWs systems since it may affect the effective area of the bed that is used for the pollutants transformations and consequently for their removal. In fact, the hydrodynamics controls the HRT and thus, the time available for pollutant in wastewater contact with microorganisms and so, for removal processes to take place (Kadlec & Knight, 1996). For example, different flow patterns can cause short circuits in the system and result in much shorter HRT, higher water velocities and less efficient wetland area used for treatment (Shrestha, 2011). HSSF-CWs are designed with a wide range of HRT, generally ranging from 2 to 20 days; however, Kadlec & Knight (1996) have been recommended a HRT between 2 and 7 days.

Hydrodynamic factors are considered to be the most important design factors in CWs, determining treatment effectiveness, because they link all of the functions in a wetland and highly affect the characteristics such as oxygen concentration, pH and redox potential (Lee *et al.,* 2009; USEPA, 2000). As we will detail later, nitrification process and substrate adsorption are crucial for the removal of nitrogen and phosphorus in HSSF-CWs. Reduced environmental conditions that characterized the HSSF could be limiting the nitrification process and so, the removal of nitrogen in this type of CWs (Song *et al.,* 2010; Zhang *et al*., 2011). However, this saturated condition produces anaerobic conditions in wetland media which in turn is favourable to denitrification. Reducted conditions can also contribute to liberate phosphorus that could be otherwise strongly held in iron and alum oxides (Ballantine & Tanner, 2010). So, water in the wetland is critical for the occurrence of specific biochemical reactions and seems to be an important controlling factor that can be determinant to the nutrient removal (Song *et al.*, 2010).

The hydraulic loading rate (HLR) refers to the loading of water volume per unit area over a specified time interval and it is defined as the volumetric averaged flow rate divided by the wetland surface area and depends on soil material, flow rate and area size (Kadlec & Knight, 1996). Too high HLR has been reported has a main limiting factor of treatment processes in HSSF wetland system. Commonly accepted ranges for HLR range from 0.2 to 3.0 $cm.day^{-1}$ (Chouinard *et al.*, 2014), despite, according to the same authors, for colder climates higher values for HLR are suggested (between 1 to 2 $cm.day^{-1}$). However, others authors such as Crites (1994) and Kadlec & Knight (1996) have suggested that the HLR value may be higher and vary from 8 to 30 $cm.day^{-1}$, especially when it seeks to promote the nitrification/denitrification process. In fact, in some studies it was found that the highest rate of nitrogen removal occurred in HSSF systems which HLR raging between 10-30 $cm.day^{-1}$ (Spieles & Mitsch, 2000; Thorén *et al.*, 2004).

2.4. Pollutant removal mechanisms in HSSF-CWs

The overall wastewater generation rate varies significantly from country to country and in Portugal it is about 155 liters per capita per day (including stormwater) (ERSAR, 2013). Wastewaters are usually characterized by physical, chemical and microbiological parameters and according the concentrations of pollutants, the strength of domestic wastewater can be categorized as strong, medium, or weak (Metcalf & Eddy, 2003) as shown in Table 2.4. In the same table are also shown the characteristic values for Portugal (Marecos do Monte & Albuquerque, 2010).

Table 2.4 Typical composition of untreated domestic wastewater

Water Quality Parameter		Unit	Concentration*			Concentration in Portugal**	
			Low strength	Medium strength	High strength	Range of variation	Typical Value
Total Solids (TS)		$mg.L^{-1}$	390	720	1230	980 - 60	715
Dissolved Solids (DS)	Total	$mg.L^{-1}$	270	500	860	210 - 720	550
	Fixed		160	300	520	-	-
	Volatile		110	200	340	-	-
Suspended Solids (SS)	Total	$mg.L^{-1}$	120	210	400	90 - 430	190
	Fixed		25	50	85	9 - 24	16
	Volatile		95	160	315	34 - 109	72
Settleable Solids		$mg.L^{-1}$	5	10	20	-	-
BOD_5, 20ºC		$mg.L^{-1}\ O_2$	110	190	350	444 - 1338	290
COD		$mg.L^{-1}\ O_2$	250	430	600	746 - 1946	608
Total Nitrogen (TN)		$mg.L^{-1}N$	20	40	70	53 - 88	60
Organic nitrogen (Org-N)		$mg.L^{-1}N$	8	15	25	12 - 16	14
Ammonia nitrogen(NH_4-N)		$mg.L^{-1}NH_4$	12	25	45	32 - 81	38
Nitrites (NO_2-N)		$mg.L^{-1}\ NO_2^-$	0	0	0	Traces – 1.3	0,19
Nitrates (NO-N)		$mg.L^{-1}NO_3^-$	0	0	0	Traces – 3.3	0,5
Total Phosphorus (TP)		$mg.L^{-1}$ P	4	7	12	3.5 - 13	8,5
Organic Phosphorus (Org-P)		$mg.L^{-1}P$	1	2	4	-	-
Inorganic Phosphorus(PO_4-P)		$mg.L^{-1}PO_4^{3-}$	3	5	10	-	9
Chlorides		$mg.L^{-1}$	30	50	90	120 - 136	128
Sulphate		$mg.L^{-1}$	20	30	50	42 - 75	47
Oil and grease		$mg.L^{-1}$	50	90	100	39 - 475	115
Alkalinity		$mg.L^{-1}CaCO_3$	50	100	200	-	-
Total coliform		NMP.100 mL^{-1}	10^6 -10^8	10^7 – 10^9	10^7 - 10^{10}	-	-
Faecal coliform		NMP.100 mL^{-1}	10^3 -10^5	10^4 – 10^6	10^5– 10^8	-	-

*Adapted from Metcalf & Eddy, 2003; **Adapted from Marecos do Monte & Albuquerque, 2010

The organic compounds can be measured as biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), and total organic carbon (TOC). A typical municipal wastewater contains an organic matter between 500 to 1000 $mg.L^{-1}$ as COD and 300 to 600 as BOD_5, with a COD/BOD_5 ratio of about 1.3 to 1.6. Municipal wastewaters normally contains from 300 to 400 $mg.L^{-1}$ of TSS, which generally are reduced to approximatlely 100 $mg.L^{-1}$ after primary treatment (Metcalf & Eddy, 2003).

Regarding to nitrogen compounds, in municipal wastewaters this element occurs as organic nitrogen (Org.-N), ammonium ion (NH_4^+-N), nitrite (NO_2^--N) or nitrate (NO_3^--N). Municipal wastewaters, usually, contains from 30 to 80 $mg.L^{-1}$ of nitrogen, mainly in the form of organic-N and NH_4^+-N (Metcalf & Eddy, 2003). According the same author the phosphorus in wastewaters is usually present as orthophosphate, polyphosphate or organic phosphorus and in a range between 6 to 10 mg.L^{-1}. In addition to those compounds, municipal wastewaters contain a great variety of microorganisms associated with the disposal of excreta (*e.g.* bacteria, viruses, fungi, protozoa and helminths), which some of them must be pathogenic and responsible for diseases such as cholera, hepatitis A and gastroenteritis.

All these contaminants when they are discharged into the receiving water bodies can place several health hazards to the general public, aquatic organisms, and the environment. Generally, sewage discharges is even considered a main cause of contamination of water resources around the world, which shall be responsible for destabilization of aquatic ecosystems. In fact, the discharge of wastewaters with high BOD can contribute to deplete oxygen in receiving waters due their microbial degradation and often also result in huge changes that could have significant consequences for biotic and abiotic components of aquatic ecosystem. Also the risks of eutrophication process in natural water bodies, associated to harmful algal blooms, have posed a significant challenge to fisheries, public health, and economies and they are often a result of discharge of wastewater rich in organic matter and/or nitrogen and phosphorus compounds, and so, it's of great importance its removal to minimize such impacts on natural water bodies.

In order to protect public health and prevent those negative environmental impacts, and for the assurance of an effective water quality management, ultimate aim of the EU Water Framework Directive (Directive 2000/60/EC of 23 October 2000), appropriate wastewater treatment strategies are crucial. As mentioned above, CWs are engineered systems that have a higher rate of biological activity and that have been designed and constructed to take advantage of many of the processes that occur in natural wetlands for transform some of the common pollutants presenting in wastewaters into harmless by-products (Kadlec & Wallace, 2009).

Major design parameters, removal mechanisms and treatment performance of HSSF-CWs have been reviewed by numerous authors (Wallace & Knight, 2006; Vymazal & Kröpfelová, 2008; Kadlec & Wallace, 2009; Mander & Mitsch, 2009), however, these processes in HSSF-CWs are not well understood because they are complex and less controllable and have variable reaction rates so, they have been often considered as "black boxes" (USEPA, 2000). According to García *et al.* (2010) there is no single pathway that can describe the complete range of processes involved in the removal of a given contaminant in HSSF-CWs. However, to improve the efficiency of this eco-friendly and sustainable technology it is essential the understanding the multiple interactions that occur within the system and the main processes responsible for wastewater treatment.

The most important removal mechanisms that occur in HSSF-CWs include liquid/solid separations (e.g. gravity separation, filtration, adsorption and volatilization) and several transformations which can involve both chemical (e.g. oxidation/reduction reactions, flocculation, acid/base reactions, and precipitation) and biochemical reactions that can occur under aerobic, anoxic or anaerobic conditions (IWA, 2000; USEPA, 2000; Vymazal, 2005).

Anaerobic metabolism seems dominate within the bed of HSSF-CWs, and it is expected that aerobic respiration is limited to the upper zone of the wetland bed and to thin layer around the roots. The mainly mechanisms by which HSSF-CWs remove the common contaminants presenting in wastewaters and that are object of the present study, are shown in Table 2.5 and a description of the processes and performances involved on removal in HSSF-CWs will be also discussed into the following sections.

Table 2.5 Main removal mechanisms of certain pollutants in HSSF- CWs (Adapted from Kadlec & Wallace, 2009; Knowles *et al.*, 2011; Bodin, 2013)

Water Quality Parameter	Type of removal mechanisms		
	Physical	**Chemical**	**Biological**
Suspended solids	Filtration, sedimentation, interception		
Organic matter (either BOD or COD)	Filtration and sedimentation of particulate Organic matter		Microbial degradation (aerobic and anaerobic)
Nitrogenous compounds (organic-N, NH_4^+, NO_3^-, NO_2^-)	Filtration and sedimentation of particulate organic nitrogen	Precipitation; adsorption; volatilization	Microbial uptake; plant uptake; Microbial transformation (e.g. ammonification; nitrification/denitrification).
Phosphoric compounds (Inorganic and organic P)	Filtration and sedimentation of organic P	Precipitation; adsorption	Microbial uptake; plant uptake
Pathogens (bacteria, viruses, protozoa, helminths)	Filtration;sedimentation	Adsorption	Natural die-off; microbial predation and exposure to toxins

2.4.1 Total suspended solids removal

Due to low velocity, long residence time, generally several days and large surface area of the media in HSSF-CWs wetlands, they have demonstrated to be effective in removing settleable or floatable solids presenting in wastewaters (Mander & Mitsch, 2009; Vymazal, 2010). This type of wetlands removes TSS primarily through a physical process of entrapment by filtration/interception and also by gravitational discrete and flocculent sedimentation of solid particles within the interstitial spaces between particles media (Table 2.5) (Karathanasis *et al.*, 2003; Garcia & Corzo, 2008).

Removal percentages for TSS in HSSF-CWs wetlands typically range from 70.0% to 95.0% when applied for secondary treatment (Wallace & Knight, 2006; Vymazal, 2009; Abou-Elela *et al.,* 2013). Adom *et al*., (2002) have reported a TSS reduction of 80% (70 g $TSS.m^{-2}.day^{-1}$), while

Jayakumar & Dandingi, (2002) and Kaseva *et al*. (2002) mentioned a TSS removal of 90% and 91.5% (9.728 g TSS $m^{-2}.day^{-1}$), respectively. Also a study on solids accumulation in six full-scale CWs used to treat municipal wastewater in the province of Lleida, Catalonia, Spain showed mean removal rates of TSS from 85 to 91% (Caselles-Osorio & Garcia, 2007), while Vymazal *et al.* (2006) in a review study, based in a worldwide experience of treatment efficiency of HSSF-CWs reported for these systems an average removal efficiency of 83.1% for TSS. According to Vymazal & Kröpfelová (2008), HSSF-CWs have been used for domestic wastewater treatment after primary settling with good results on TSS quality of the effluent whose value is generally less than 30 $mg.L^{-1}$ and therefore meeting the limit established by the Portuguese legislation (≤ 35 $mg.L^{-1}$).

One of the main parameters that seems affecting TSS removal in a HSSF-CWs are the hydraulic characteristics of the substrate. In fact, for an adequate filtration and sedimentation to occur, the hydraulic conductivity of the bed must be large enough to allow the wastewater to contact the media. Reed & Brown (1995) have come to a relation based in a regression analysis, between TSS removal and hydraulic loading rate based on data from 14 operating HSSF-CWs:

$$C_e = C_o\,[0.1058 + 0.0011\ HLR]$$

Where: C_e = effluent TSS concentration ($mg.L^{-1}$)

C_o = influent TSS concentration ($mg.L^{-1}$)

HLR = hydraulic loading rate ($cm.day^{-1}$)

At increased HLRs, TSS removal tends to decrease because solid particles have higher probability of being entrained due to the increased of flow velocities. However, Weerakoon *et al.* (2013) in a study on impact of HLR on pollutants removal in HSSF systems have observed that these types of CWs have good buffering capacity under varying flow conditions between 2.5 and 25 $cm.day^{-1}$ HLR range, which could indicate that this type of technology may be particularly suitable for places with high flow fluctuations.

Plants are also reported to have a positive effect on TSS removal due its role on reducing water velocity and maybe by promoting settling and filtration in the root system, as we have previously mentioned. Karathanasis *et al.* (2003) have report that average percentage removal of TSS was significantly higher in vegetated systems (88-90%) than in unplanted wetlands (46%) and they explained these results both due to more effective filtration and sedimentation in planted systems due the rooting biomass and by increased attachment surface area for microbial growth, which also contributes for the removal of the organic portion of TSS through microbial decomposition. They have also observed that these differences in TSS removal efficiencies, between planted and unplanted units, have persisted throughout all seasons, which is consistent with the results obtainable by several others authors (LLorens *et al*., 2009; Garfi *et al.*, 2012; Hijosa-Valsero *et al.*, 2012).

These results seem to confirm that TSS removal is mainly a physical process of filtration and sedimentation, and therefore less sensitive to temperature or seasonal conditions, although it can be observed a slight improvement in removal rates in the warmest season, mainly due to the increase in HRT caused by the increases of evapotranspiration and the decrease of rainfall (Dušek *et al*., 2008).

The TSS removal is localized, and seems to take place, mainly in the first few meters beyond the inlet. It was found that 60-75% of solids removal in these wetland systems occur in the first one third of the wetland (Trang *et al*., 2010). So, over time, the accumulation of these materials in this zone can contribute to obstruction of the pore spaces of the filter media, which is considered one of the major operational problems of HSSF-CWs and is known as clogging (Knowles *et al.*, 2011). In fact, some studies reported a positive correlation between TSS loading rates and the occurrence of clogging problems on the system (Rousseau *et al.,* 2004). Caselles-Osorio & Garcia (2007), in Spain, in a study with six full-scale HSSF-CWs that treat urban wastewater, observed a positive correlation between solids accumulations rates and the TSS and COD loading rates applied to the systems and a inverse correlation between hydraulic conductivity of the media and the amount of accumulated solids.

The clogging is a complex process that has a negative impact not only on porosity of the bed, but especially it can contribute to unfavourable changes in the hydrodynamic of the system, such as short-circuiting and gradual decrease in HRT (Trang *et al*., 2010). As the porosity decreases, the real volume also changes and this can result in variable and decreasing treatment performance, because the wastewater pass faster than the projected theoretical HRT, resulting in a lower biodegradation rate of the pollutants as a consequence a smaller contact time with biofilm (Knowles *et al.*, 2011). This problem could also cause severe vegetation dieback due to oxygen stress in this zone.

In the most severe situations of clogging, the lifetime of the wetland treatment system may be reduced to a few years, according to Nivala *et al*. (2012) by one-tenth, and some authors have found that this problem tends to become worse when the TSS concentration in the influent is above 50 $mg.L^{-1}$ (Garcia & Corzo, 2008; Knowles *et al.,* 2011). Therefore, in order to avoid clogging, some authors considered that TSS loading rates on the inlet zone cross section must be lower to 20 g $TSS.m^{-2}.d^{-1}$ for HSSF-CWs (USEPA, 2000; Vymazal, 2010) and thereby, is also important the use of a primary treatment as a preliminary step for HSSF-CWs to ensure the removal of coarse and heavy solids.

2.4.2. Organic matter (BOD_5 and COD) removal

When HSSF-CWs are used as secondary treatment, the removal of organic matter is one of the objectives. The organic matter in municipal wastewater is either in solution or as particulate form, although according to Marani *et al.* (2004), more than 60% of organic matter present in domestic wastewater is in particulate form and slightly under a half of this organic compounds is large enough (> 100 µm) to rapidly be removed by sedimentation and /or filtration in the porous of wetland bed. Vymazal (2010) reported that total organic matter tend to decrease rapidly close to the inlet zone and little additional removal occurs beyond this zone, due to the fact that great part of the organic matter in domestic wastewater is retained as TSS in the inlet zone.

The soluble organic compounds (<0.001 µm) are removed more slowly and along the entire length of the bed by attached microorganisms that form the biofilm and by suspended microbial communities that use organic carbon as a source of energy (García *et al.,* 2005; Li *et al.,* 2008). The soluble organic matter is present in influent, but is also produced by heterotrophic microorganisms through decomposition of particulate organic matter removed by filtration and sedimentation (Vymazal & Kröpfelová, 2008). Thus, the removal pathways of organic matter in HSSF-CWs involve physic-chemical removal and different biochemical processes occurring in different zones of the wetland.

As in the case of TSS, HSSF-CWs systems also have their internal organic matter loads which results from growth, dieback and decomposition of biomass produced by wetland plants and many of this organic matter seems to be recalcitrant compounds and therefore, they are slowly decomposed due to lignocellulose constitution (Tanner *et al.*, 1998). Biofilms are also at the origin of decay products that are highly resistant to biological degradation reactions (Vymazal, 2010).This primary production in the wetland itself is represented as C* and also contribute to form a background concentration of organic matter that can influence the performance of the system, especially if wetland is planned to treat wastewater having low concentration of organic matter (García *et al*. 2010). This background organic matter, in terms of BOD, is often assumed to be around 3 mg $O_2.L^{-1}$ (Rousseau *et al.*, 2004).

Biological removal of organic matter includes a diversity of complex biochemical reactions that are dependent on the environmental conditions of a particular system due to its influence in the activity of specific groups of microorganisms. According to Paredes *et al.* (2007) the importance of these biochemical pathways seems to be dependent primarily on the redox conditions (anaerobic, anoxic and/or aerobic) that are different for specific microbial processes involved in organic mineralization and so, in organic removal rates. Generally, it has been found that the prevalence of high redox potential promote oxidizing conditions (from +250 to +700 mV) which ensurs aerobic decomposition that is more efficient in removing organic matter than anaerobic degradation pathways that tend to occur at lower redox potential (+250 to -400 mV) (Faulwetter *et al*., 2009; Hijosa-Valsero *et al*., 2012).

In a particular HSSF wetland, the redox potentials conditions depend on several factors, mainly related with design and mode of operation of the system such as depth of the bed, hydraulic and organic loading rates, feeding strategy and natural/artificial aeration. Within HSSF systems there are also zones with different redox potential, at macro and micro-scales, which can present short and long-term temporal variability and that could be affect and explained the most important pollutant removal mechanisms that largely occur in this type of wetland system (LLorens *et al.,* 2009).

Generally, the redox potential in these wetlands tend to increase from the inlet to the outlet zone due mainly to the progressive removal of organic matter, but also to the gradual biodegradation of other pollutants and usually also decreases from surface to deep layer, with higher values generally being observed on the surface zone (5-20 cm) due to passive oxygen diffusion and root oxygen release (Headley *et al.,* 2005; Faulwetter *et al.,* 2009). García *et al.* (2003) observed at full-scale HSSF-CWs that redox potentials values varied between -200 mV at the inlet zone and +150 mV at outlet, and they also verified that there are a tendency for redox potential decreased with depth, which have been attributed to small importance either of surface aeration by directly diffusion of atmospheric oxygen or oxygen release by root system at deeper layers of filter bed.

So, in HSSF-CWs the main degradation process of organic matter seems to be carried out by a consortium of microorganisms either facultative or obligate anaerobic heterotrophic bacteria that works synergistically and involves multi-steps processes and alternative biochemical pathways (Figure 2.10) (Chavan & Dhulap, 2012). After hydrolysis of complex organic compounds to simple organic monomers, these compounds are converted by heterotrophic acidogenic bacteria into volatile short-chain fatty acids (e.g. acetic, propionic, butyric, lactic), alcohols and the gases CO_2 and H_2 (Cooper *et al.*, 1996; Vymazal *et al.*, 1998; Mitsch and Gosselink, 2000).

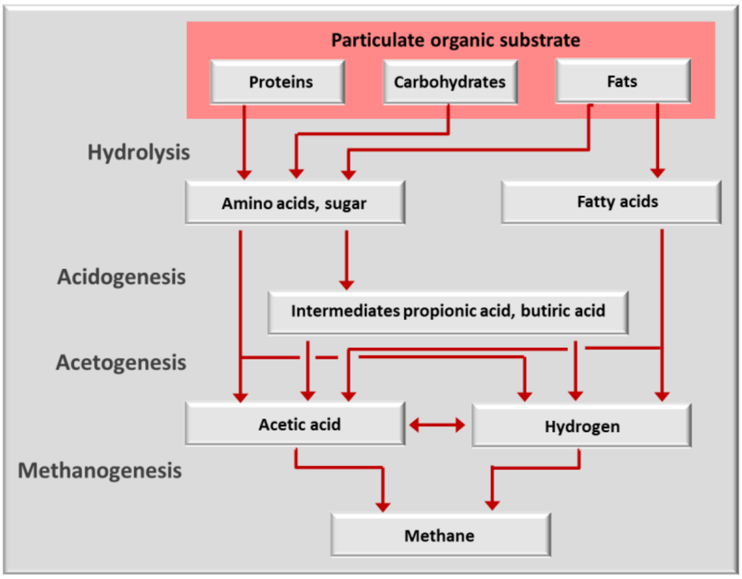

Figure 2.10 A scheme of anaerobic degradation involving multiple biochemical reactions (Serna, 2009)

The end-products of the first step and others intermediates compounds are used as substrate by strictly anaerobic bacteria that can use sulphate, a normal constituent of many wastewaters, as final electron acceptor (sulphate reduction organisms) or by methane-forming bacteria that produce methane and carbon dioxide through biochemical reactions known as methanogenesis

(Vymazal *et al*., 1998). Both groups seem to play an important role in the oxidation and removal of organic matter and carbon in HSSF-CWs (Faulwetter *et al*., 2009; Meng *et al.,* 2014).

Caselles-Osorio & García (2007) have concluded that sulphate reduction and methanogenesis seemed to be the most effective metabolic pathways for removing organic matter in HSSF-CWs, and they also observed that the highest COD removal efficiencies were obtained in the absence of sulphates, having attributed this fact to the potential toxic effects of sulphide release during the activity of sulphate reducing bacteria for wetland plants and for certain microbial activities. Methanogens and sulphate reducing bacteria require environments with similar redox potential and use the same types of electron donor (*e.g*. hydrogen, methanol and acid acetic).

According the same authors, for a sulphate ratio (expressed as COD: S) lower than 1.5, sulphate reducing bacteria seems to be able to outcompete methanogens, while for a ratio more than 6, methanogens tend to predominate. Methane-formers are most sensitive to pH conditions than the acid-forming bacteria, only operating at pH values in the range between 6.5 and 7.5 (Vymazal *et al*., 1998; García *et al*., 2010). So, an over production of acid in first step can quickly result in a lowering of the pH and then the action of methanogenic bacteria can stop which can lead to release of unpleasant odors from the HSSF-CWs.

García *et al.* (2005) observed that the relative importance of different mechanisms responsible for the degradation of organic matter in the HSSF is also dependent on the depth of the bed, and found that methanogenesis and sulphate reduction are more important to the removal of organic matter in a 0.5 m depth HSSF than in those with a depth of 0.27 m. They attributed this fact to the decreased of DO concentration and to the vertical profile of redox potentials gradients with depth as previously mentioned. As well as sulphate reduction, also denitrification process contributes for organic oxidation because the microorganisms involved in this process are facultative heterotrophic bacteria (Wieβner *et al*., 2005).

According to Faulwetter *et al.* (2009), generally, 25% of carbon removal has been attributed to the sulphate reducing bacteria community, while García *et al.* (2010) stated that methanogenesis is responsible for removing the most significant amounts of organic matter from wastewater in HSSF, which is in agreement with findings by LLorens *et al.* (2011) in a study on the relative contribution of different microbial reactions to organic matter (COD) removal in a HSSF-CW treating urban wastewater. In fact, they observed that anaerobic processes were more widespread in the simulated wetland and contributed to a higher COD removal rate (72-79%) than anoxic (0-1%) or aerobic reactions (20-27%) have contributed. In all the cases tested, the reaction that most contributed to COD removal was methanogenesis (58-73%).

Several studies carried out in various regions of the world have shown that HSSF-CWs usually have very good treatment efficiencies regarding the removal of organic matter, although the performance of each specific system depend of different external and internal factors, such as the design and operating parameters, organic loading rate of organic matter, temperatures, plant species and redox conditions that can control the competition between the different microbial consortia responsible for the decomposition of different organic compound (Akratos &Tsihrintzis, 2007; Albuquerque *et al*., 2009a; LLorens *et al*., 2011).

Generally, the literature reported a removal percentage ranging between 71-94% and 69-89% for BOD_5 and COD, respectively (Tuszynska & Obarska-Pempkowiak, 2008; García *et al*., 2010). Vymazal (2005) reported a treatment efficiency of HSSF based on data from worldwide experience about 85% for BOD_5 and 75% for COD. Jayakumar & Dandigi, (2002), found a BOD_5 reduction of 87.5%, while Kaseva *et al*., (2002) and Senzia *et al.*, (2003) observed values of 75% (5.83 $gBOD_5.m^{-2}.day^{-1}$) and 82.2% (4.039 $gBOD_5.m^{-2}.day^{-1}$), respectively. García *et al*. (2004) have reported that in order to obtain a BOD_5 removal of 90%, for urban wastewater, an organic surface loading of 200 $Kg.ha^{-1}.day^{-1}$ should not be exceeded. In Portugal, the data available on the effectiveness of HSSF-CWs points to removal rates of the same order of magnitude.

The organic matter removal efficiency along the HSSF-CWs seems to be also influenced by the biodegradability of organic matter present in wastewaters due to its crucial effects in microbial degradation process. In fact, labile (e.g. proteins, carbohydrates) and recalcitrant (*e.g.* hemicellulose, lignin) fractions of organic matter are present within the wetlands can might influence the rate at which this organic matter will be degraded by microorganisms. However, Caselles-Osorio *et al*. (2007) in a nine-month study in two experimental HSSF-CWs for evaluating the impact of two type of organic substrates (glucose - dissolved and readily biodegradable; starch – particulate and slowly biodegradable) on COD removal efficiency wetlands operated under different HRT, observed that at same low organic loading rate (6 $gCOD.m^{-2}.day^{-1}$) the HSSF-CWs have similar COD removal rates, which ranged from 85 to 95%, despite the glucose stimulate greater biofilm production due to its fast biodegradation. In the same study, the authors evaluated the effect of organic loading rate and they have observed that, there were no significant differences in COD removal between the high (22 $gCOD.m^{-2}.day^{-1}$) and low (6 $gCOD.m^{-2}.day^{-1}$) organic loading rates.

However, others studies reported that higher organic removal rate (in terms of percentage or mass surface rate) tends to occur when increasing the organic input load applied to the system (Headley *et al.*, 2005; Chazarenc *et al.*, 2007; Saeed & Sun, 2012). Amado *et al*., (2012) in a monitoring campaign of 30 months in a full-scale LECA-based HSSF-CW located in an Interior Region of Portugal reported a significant linear relationship between applied and removed loads for organic matter (R^2 (BOD) = 0.91; R^2 (COD) =0.73). Also Frazer-Williams (2010) reported that effluent organic load (BOD and COD) showed strong positive correlations (R^2 =0.86 and R^2=0.91, respectively) with influent organic loading in a HSSF-CW. Similar correlations for COD were found by Avsara *et al*., (2007), Albuquerque *et al.* (2009a) and also by Vymazal (2002) for a study on 44 HSSF systems in the Czech Republic. These results indicate that HSSF systems seem to have a good response to changes in incoming loads, which are also consistent with results presented by Galvão & Matos (2012).

Nevertheless, organic overloading may contribute for some negative effects; one of them is the potential fast accumulation of organic matter and/or a high rate of microbial biomass growth at inlet zone which can contribute to clogging problems and in order to prevent this phenomenon, it has been recommended that organic loading rates should not exceed 6 $gBOD_5.m^{-2}.day^{-1}$ in HSSF-CWs as we have already mentioned (USEPA, 2000; Knowles *et al.*, 2011).

The efficiency of HSSF wetlands also depend on other operational factors like HRT, even if has been observed a good removal rates of organic matter at short HRTs. Akratos & Tsihrintzis (2007) in a study on the effect of HRT (i.e. 6,8,14 and 20 days) on removal efficiency of pilot-scale HSSF-CWs have concluded that a HRT above 8 days is adequate for relatively high removal of organic matter and that BOD removal efficiency increased from 76.5% to 81.9% when HRT increased from 6 to 8 days. García *et al.* (2005) in a HSSF system involving several wetland cells, which received different HLRs (0.02, 0.036, and 0.045 $m.d^{-1}$), found that HLR was controlling the effluent concentrations of COD and BOD5, showing higher removal efficiency at lower loads, which is consistent with results obtained by Amado *et al*. (2012).

The effect of season and temperatures on organic matter removal efficiency in the CWs have been reported by some studies, with the worst performance occurring usually during the winter and having attributed this fact to the decrease in daily average temperatures (Song *et al*., 2006; Taylor *et al.,* 2011; Stefanakis & Tsihrintzis, 2012). In fact, temperature can be a critical factor in biological removal mechanisms in wetland systems due to its influence both at its effect on the metabolism of microorganism and composition of the microbial community as due its effect at the level of development of the plants and hence its potential oxygen release rate (Garfi *et al.*, 2012; Gikas & Tsihrintzis, 2014). In general, higher temperatures (> 20 ºC) result in higher biological activity and microbial growth rates, while lower temperatures (< 10 ºC) will restrict the biological activity and seems to lead to accumulation of organic matter and others pollutants in CWs (Faulwetter *et al.,* 2009).

Mancilla-Villalobos *et al.* (2013) have compared the removal data for each season (winter/spring, autumn and summer), and they have observed that the group winter/spring was the one that showed the lowest total COD removal and they attributed this fact to the decrease in daily average temperatures between the seasons. Similar results were obtained by Hijosa-Valsero *et al.* (2012). Also, Garfi *et al.* (2012) in a continental Mediterranean climate region of Spain have observed that BOD removal efficiency followed seasonal trends ranged from 96.2% in summer to 65% in winter. The latter authors also observed that the dependence of pollutant removal efficiency on season is more emphasized in continental climate regions than in Mediterranean due to more severe winters in continental regions.

Akratos &Tsihrintzis (2007) also had observed that BOD and COD removal are dependent of temperature, but they have not found a significant correlation between the two variables. This result may be related to the fact that heterotrophic bacteria responsible by degradation of organic matter seems to continue active even for temperatures as low as 5 ºC (Vymazal, 2002). However, others studies indicate that organic matter removal efficiency is independent of season (Brix & Arias, 2005).

Some studies have also concluded that plants can also affect the influence of temperature on removal efficiencies of pollutants in HSSF-CWs. In fact, Taylor *et al.* (2011), observed that COD removal in unplanted system tend to decrease at colder temperature, but this seems to have had little influence on planted wetland systems. Also Allen *et al.* (2002) in a batch wetland microcosms have found that the effects of plants on performance of wetland systems tend to be great during coldest periods and they attributed this fact to the effect of plants in redox conditions, although this effect vary according to different species. In fact, according to this study, it seems that in colder seasons the plants can reduced their root respiration rate due to cold temperature and so, during the dormancy period they seem to release more oxygen for root zone, which could induce greater microbial metabolic activity.

However, it is important to note that this effect can be very diluted in full-scale systems due to other equally important factors such as the potential application of high hydraulic and organic loads, the tendency to increase the HLR due to rainfall, senescence of plants and mainly the continuous flow operation typical in HSSF-CWs, all contribute to maintain the system continuously with high oxygen demand and so, limit development of oxidizing conditions (Hijoso-Valsero *et al*., 2012; Stefanakis & Tsihrintzis, 2012). Therefore, it is unclear whether the poor winter efficiencies are due to low temperatures or are the result of the combined effect of all those factors.

The comparison of organic removal efficiency of planted HSSF-CWs and unplanted is not consensual, despite most studies have shown that systems with plants tend to exhibit higher treatment efficiency (Stottmeister *et al*., 2003; Akratos & Tsihrintzis, 2007; Brisson & Chazarenc, 2009; Taylor *et al*., 2011). This could be explained, as previously discussed in the section 2.3.2 not only by plants provide surfaces for microbial attachment, but also by increased oxygen supply to the rhizosphere, although at different rates by different species, as compared to unplanted systems, which seems to enhance the activity of aerobic heterotrophic bacteria and then contribute to increase the decomposition by this pathway of organic matter.

In fact, plants also influence redox condition due to effect on the transfer of oxygen for the substrate media promoting an oxidized layer around the root and creating a redox gradient from ranging from $E_h \sim +500$ mV near root surface to $E_h \sim -250$ mV at a distance of 1-20 mm from the root surface (Münch *et al*., 2005; Wiessner *et al*., 2005). Hijosa-Valsero *et al.* (2012) has also obtained a positively correlation between the presence of plants and redox potentials values, verifying that the plants tend to increase redox potentials, which contributed to create aerobic microsites, which is in accordance with that found by other authors (Stottmeister *et al*., 2003; Imfeld *et al.*, 2009).

Camacho *et al*. (2007) in a five continuous HSSF-CWs treating domestic wastewater evaluated the effects of different plant species (*Lythrum salicaria, Iris pseudacorus, Purple loosestrife* and

Yellow Flag) on organic removal efficiency. They observed that all of the CWs removed between 80% and 90% of the influent COD and the plants that gave rise to the best performances were the *Purple loosestrife* (90.3% COD removal) and the Yellow Flag (88.6%), while the worst performance was given by the CW without plants (80.7%). So, this study has shown that planted CWs improved COD removal compared with unplanted and also seems to suggest that different species can differently affect the performance of HSSF systems.

On the other hand, Akratos & Tsihrintzis (2007) observed that between the BOD mean removal efficiency obtained in three units (with same porous media (gravel) and different plants (cattails, common reed and unplanted)), only the value achieved for cattails unit (88.3%) are statistically different from the others units that were 84.6% for common reed and, 85.7% for unplanted unit. The higher removal efficiencies in organic matter by cattails units were attributed to its more vigorous root system. These results are consistent with those presented by other authors, who generally consider that these differences can be attributed to differences in the development of the root system of different species, which may influence the release rate of oxygen to the rhizosphere zone (Salvato *et al*., 2012).

These results have led to studies to evaluate the potential benefit of using systems with polycultures. Karathanasis *et al.* (2003) have study the effect of *Typha latifolia*, *Festuca arundinacea* and polycultures consisting mainly of *Iris pseudacorus*, *Canna x. generalis*, *Hemero callisfulva*, *Hibiscus moscheutos*, *Scirpus validus* and *Mentha spicata.* Generally, the polycultures systems seemed to provide the best and most consistent treatment for all wastewater parameters and also showed to be less susceptible to seasonal variations. They attributed this behaviour to the fact that the presence of several species can ensure a more favourable environment to a most diverse microbial population than the monoculture systems.

Also Abou-Elela *et al.* (2013) observed that HSSF-CWs planted with three species presented significantly higher removal efficiencies in comparison to those planted with only one of the plants tested (*Canna* spp., *Phragmites* spp. and *Cyperus* spp.). Karathanasis *et al.* (2003) also observed that the vegetated systems also showed a statistically greater annual removal in BOD than the unplanted systems, with a slight decline during the winter. The seasonal variations on the removal of pollutants can also be linked to the growing cycle of the plant itself, which vary over the different seasons with enhanced plant growth in spring followed by senescence and plant decay in fall and winter (Brix & Arias, 2005), as previously mentioned.

Finally, it is known that the pH affect the microbial activity and thus, the mineralization of organic matter could be more or less fast and carried out through specific microbial processes in accordance with the pH conditions. Heterotrophic production rates tend to be higher at near-neutral pH values of wastewater than that in acidic wastewater. On the other hand, the ideal pH for methane forming bacteria is 6.5–7.5 (Saeed & Sun, 2012), while the optimal pH range for denitrification activity is 6–8, thus verifying that tends to fall for values < 5 and even is absent below pH = 4 (Vymazal, 2007), two biochemical processes we have previously seen that could have particular relevance in the removal of organic matter in subsurface horizontal flow systems.

Presently some effort is being putted in triying to improve some aspects of construction and/or operating of the beds in order to increase the removal of pollutants, especially organic matter and nutrients (N, P), such as the type of feeding system of beds and the feasibility of using artificial aeration. Continuous feed is the most common technique in HSSF wetlands which results in lower redox potential and subsequently in less effective removal of aerobic organic removal (Caselles-Osorio & Garcia, 2007). However, HSSF wetlands can be operated at loading-resting cycles, in order to enhance the aerobic conditions within the wetlands, which seem to contribute for a higher performance when compared with continuous operation (Caselles-Osorio & García, 2008, Zhang *et al*., 2011).

Pedescoll *et al.*, (2011), have reported a higher performance in terms of COD removal under intermittently feeding, as opposed to the continuous one, particularly in winter (up to 50% higher than a continuously fed system). According to Allen *et al*. (2002) the batch or intermittent feed contribute to create a temporal redox variation, which may favour the removal mechanisms due to being able to create favourable conditions for the development of a robust microbial community, mainly constituted by facultative species. Also, Wallace & Knight (2006) reported a significant improvement of the system in terms of BOD removal when the HSSF beds were artificial aerated. Aerated wetlands consist of a network of drip-irrigation tubing on the bottom of the wetland cell that is connected to an air blower. However, active aeration represents an added-up cost of operation and maintenance and, until the moment, there are no clear comparative analysis on the use and benefits of active aeration in HSSF-CWs.

2.4.3 Nitrogen compounds removal

Nitrogen occurs in different oxidation states and is present in particulate and dissolved organic and inorganic forms in wastewaters (Albuquerque *et al.*, 2009a). In domestic wastewaters the most common nitrogen forms are NH_4^+-N that represents the prevailing form (at 66%-90% of TN in raw wastewater) and Org-N with little or none NO_2^--N and NO_3^--N concentrations (Metcalf & Eddy, 2003; Gajewska *et al*., 2015). However, the Org-N can be transformed into NH_4^+-N via ammonification process and so, is often included in known total Kjeldahl nitrogen (T_KN) as a potential ammonia source (Metcalf & Eddy, 2003).

Nitrogen compounds are an environmental problem of major concern because it can exert a significant negative impact in water resources due to the oxygen demand related with the ammonification process of organic nitrogen and nitrification of NH_4^+-N species, which can result in strong depletion of dissolved oxygen (DO) concentration in the receiving water bodies. Non-ionized ammoniacal nitrogen (NH_3-N) is potentially toxic to fishes and all forms of nitrogen compounds, coupled with phosphorus compound, may be at the origin of one of the most serious problems in terms of pollution of surface water resources throughout the world, the phenomenon known as eutrophication.

So, in wastewaters treatment systems it is vital to control the reduction of nitrogen compounds present in wastewaters in order to preserve the receiving bodies and in accordance with Water Framework Directive, ensure that the “ecological status” of water resources is at a level which would occur with minimal anthropogenic impact. Wetlands systems are often referred as having the ability to remove nitrogen from wastewaters, despite the data from the various studies that have been developed around the world in this field are often inconsistent and whilst some of studies have shown that CWs can be effective at reducing nutrients (Verhoeven *et al.*, 2006; Vymazal, 2007; Chung *et al*., 2008; Debing *et al*., 2010; Tanner *et al*., 2012), others reported that they have little effect or even increase nutrient concentrations in outflow (Senzia *et al.*, 2003; Sun *et al*., 2005; Mustafa *et al*., 2009).

Nitrogen removal rates reported for HSSF-CWs are variable, ranging from 90% (Søvik & Mørkved, 2008) to as low as 11% (Kuschk *et al.*, 2003). Vymazal (2005) reported 42% as TN average treatment efficiency of HSSF-CWs based on data from worldwide studies (137 systems were evaluated), while for NH_4^+-N this value was of 40% (151 systems evaluated) and for NO_3^--N they found 35% (for 70 systems evaluated). Also, based on data collected at 268 HSSF systems in Europe, the same author has concluded that the values did not differ significantly from the above range of values and found that a removal of nitrogen may range from 30 to 40%, with mean values for TN removal of 40% and 30% for the NH_4^+-N.

Nitrogen within a wetland is often interchanged between the atmosphere, organic matter and inorganic compartments through dynamic and complex processes involving several microorganisms that controll potential biochemical reactions (Table 2.5) through the production of enzymes that catalyze these reactions and organic-N, NH_4^+-N, NO_2^--N and NO_3^--N seems to be the mainly important forms involved in nitrogen cycle within wetland systems (Mayo & Mutamba, 2004; Vymazal, 2007). Gaseous nitrogen may also exist as dinitrogen (N_2), nitrous oxide (N_2O), nitric oxide (NO_2) and ammonia nitrogen (NH_3) (Vymazal, 2007). The understanding of main processes involved in those nitrogen transformations and the environmental factors that control those transformations is crucial for enhance the nitrogen removal in wetland systems.

Table 2.6 Potential biochemical reactions involved in transformation of nitrogen within wetlands systems (Adapted by Vymazal, 2007).

Process	Chemical transformation
Ammonification	Organic -$N_{(aq.)} \Rightarrow NH_3\text{-}N_{(aq.)}$
Nitrification	$NH_4^+\text{-}N_{(aq.)} + 2O_2 \Rightarrow NO_3^-\text{-}N_{(aq.)} + 2H^+ + H_2O$
Denitrification	$2NO_3^-\text{-}N_{(aq.)} \Rightarrow 2NO_2^-\text{-}N_{(aq.)} \Rightarrow 2NO_{(g)} \Rightarrow N_2O_{(g)} \Rightarrow N_{2\,(g)}$
Volatilization	$NH_4^+\text{-}N_{(aq.)}, NH_3\text{-}N_{(aq.)} \Rightarrow NH_3\text{-}N_{(g)}$
N_2 Fixation	$N_{2\,(g)} \Rightarrow$ Organic-$N_{(aq.)}, NH_3\text{-}N_{(aq.)}$
Biological assimilation	$NH_3\text{-}N_{(aq.)}, NO_3^-\text{-}N_{(aq.)} \Rightarrow$ Organic-$N_{(aq.)}$
Ammonia adsorption	$NH_4^+\text{-}N_{(aq.)} \Rightarrow NH_4^+\text{-}N_{(s)}$
Anammox	$NH_4^+\text{-}N_{(aq.)} + NO_2^-\text{-}N_{(aq.)} \Rightarrow N_{2\,(g)} + 2H_2O$
Dissimilatory nitrate reduction	$2NO_3^-\text{-}N_{(aq.)} \Rightarrow NH_3\text{-}N_{(aq.)}$
Organic nitrogen burial	fractions of the organic nitrogen incorporated in detritus in a wetland

The removal of nitrogen from influent in HSSF-CWs seems to take place mainly through biological ammonification and nitrification/denitrification processes (Langergraber, 2013; Shelef *et al.,* 2013; Wu *et al*., 2014; Gajewska *et al*., 2015). However, in these systems there are others mechanisms that can also contributed to global nitrogen removal, although they seem to be of minus important in HSSF systems, such as microbial assimilation, plant uptake, adsorption and ammonia volatilization (Vymazal, 2010; Coban *et al*., 2015). Nowadays it is also considered that besides the transformations of nitrogen controlled by classical nitrogen pathways, in wetland systems could also occur others mechanisms that may contribute to nitrogen removal, especially an alternative that includes Anaerobic Ammonium Oxidation known as Anammox, by which the NH_4^+-N can be reduced directly to N_2 using NO_2^--N or NO_3^--N as an electron donor (Lee *et al*., 2009; Tao & Wang, 2009; Wang & Li, 2011).

The ammonification constitutes a complex biochemical process in which Org-N (RN) is converted into NH_4^+-N (Figure 2.11) and is usually considered a relatively fast transformation which constitutes the first step in mineralization of organic nitrogen present in wastewater and so, essential for the global nitrogen removal from domestic wastewaters (Meng *et al*., 2014; Redmond *et al.,* 2014). This complex biochemical process include breakdown of organic tissues by microorganisms heterotrophies, in particular by exoenzmyes that deaminate these compounds to simpler amino-acids (monomers) and then to NH_4^+-N and hydrolysis of urea and uric acids through a complex energy-releasing (Kadlec & Knight, 1996). The energy released is used by microorganisms for growth and part of the ammonia is incorporated into microbial biomass. A typical ammonification reaction is the following (Kadlec & Wallace, 2009):

$$NH_2CONH_2 + H_20 \rightarrow 2NH_3 + CO_2 + \text{energy}$$

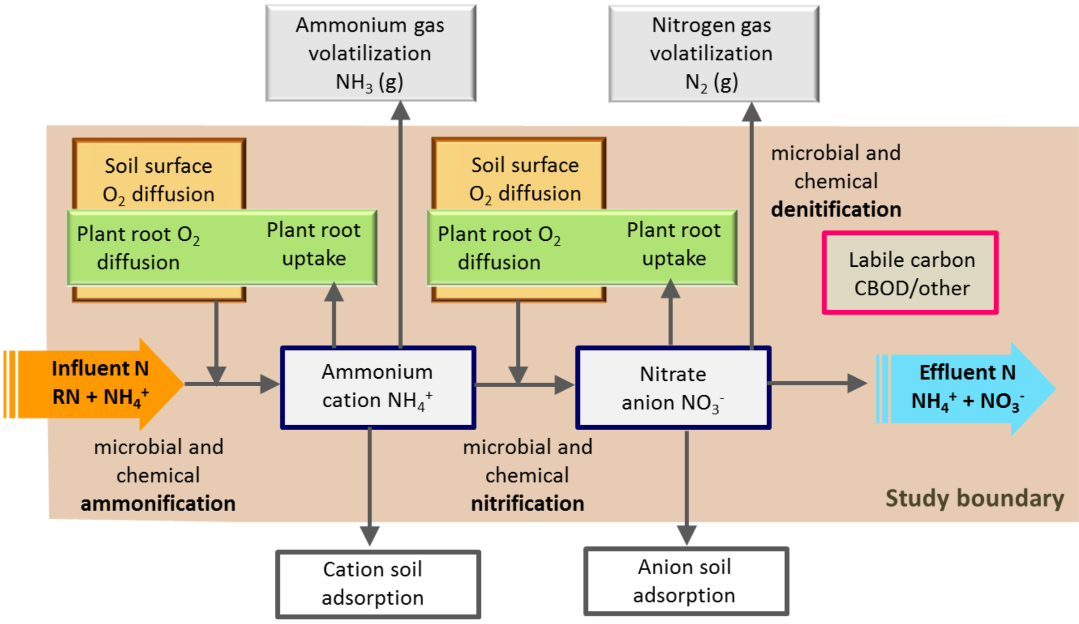

Figure 2.11 Schematic diagram showing the major nitrogen transformations in subsurface flow constructed wetlands where the dashed line represents the boundary of the granular medium (adapted from Fuchs, 2009)

A wide range of ammonification rates are reported in the literature, with values ranging between 0.004 and 0.053 gN $m^{-2}d^{-1}$ (Tanner *et al.*, 2002; Kadlec & Wallace, 2009), probably due to the complex processes which occur during treatment wetland systems and also because of the multiple factors which might affect the process. In fact, according to Cooper *et al.* (1996) DO concentrations of above 1 $mg.L^{-1}$ are required below which oxygen becomes the limiting factor for growth of aerobic microorganisms. Ammonification rates also appear to be dependent on temperature, pH, and C/N ratio between others factors (Vymazal & Kröpfelová, 2008). According to Vymazal *et al.* (1998) the optimal ammonification temperature is reported to be 40-60 ºC and they conclude from the literature data that the rate of aerobic ammonification doubles with a temperature increase of 10ºC, while optimal pH is between 6.5 and 8.5.

For conventional wastewater treatment processes, when COD/BOD_5 is below 2 and in the same time BOD_5/TN is over 4, the wastewater is easily degradable and the efficiency for both organic matter and nitrogen removal is over 90 % (Miksch & Sikora, 2010 cit. by Gajewska *et al.*, 2015). However, in case of treatment wetland, it has been considered that in order to ensure an effective nitrification the amount of readily degradable organic matter to T_KN (BOD_5/T_KN) could be <1, probably due to the capacity of wetland systems in providing endogenous sources of biodegradable organic matter (Kadlec & Wallace 2009).

Gajewska *et al.* (2015), in a study on the impact of influent wastewater quality (BOD_5/N) on nitrogen removal rates in multistage treatment wetlands in which have compared the long-term performances obtained at several systems operating in Europe (North and South) and North Africa, observed that the statistical analysis performed on all the studied facilities have shown that these systems are capable to ensure sufficient removal of both organic and nitrogen even in unfavourable proportions of macronutrients (C and N). However, they also found that values of the ratio BOD_5/TN in the range 1.5-2.5 have ensured the highest nitrogen removal rates in the observed monitoring periods.

The NH_4^+-N present in wastewater and/or formed by ammonification within HSSF-CWs has a removal rate that has shown to be widely variable and some studies even show that sometimes the HSSF-CWs systems produce ammonia instead of removing it (Kuschk *et al.*, 2003; Vymazal, 2007). This fact may be explained, by the decomposition either of the organic nitrogen present in the wastewater or the organic nitrogen present within the system due to microbial die-off and plant decay, which contributes to increase the concentration of NH_4^+-N and due to anaerobic conditions at the bed, which does not allowing complete ammonia oxidation. In addition, kinetic ammonification of organic nitrogen proceeds more rapidly than nitrification, thus creating the potential for an increase in ammonium concentration in the outlet (Kadlec & Wallace, 2009).

Nitrification seems to be one of the most important mechanisms in removing the concentration of NH_4^+-N in wastewater treatment, including at HSSF-CWs, and is typically associated with chemolithoautotrophic bacteria, i.e. the nitrifying bacteria derive energy for their growth from the oxidation of ammonia and/or nitrite, while carbon dioxide (CO_2) or carbonates is used as a carbon source for synthesis of new cells (Lee *et al*., 2009) (Figure 2.11). As it is an aerobic chemo-autotrophic microbial process and as we have already seen, oxygen diffusion is generally limited in HSSF-CWs so, this mechanism might become the limiting factor for controlling nitrogen removal in these types of wetland systems.

The first step of nitrification is ammonia oxidation to nitrite mediated by strictly aerobic chemolithoautotrophic bacteria, known as ammonium oxidizing bacteria (AOB), include species belonging to β-Proteobacteria include the genera *Nitrosospira, Nitrosovibrio, Nitrosolubus, Nitrosococcus* and *Nitrosomonas*. Bothe *et al.* (2000) observed that besides bacteria of the genus *Nitrosomonas*, the major AOB in conventional treatment systems, in wetland systems also occurs others AOB genera such as *Nitrosospira* and *Nitrosococcus* and according to Austin *et al.* (2003) *Nitrosospira* bacteria is much more prevalent than *Nitrosomonas* in treatment wetland systems. This step is catalysed by ammonia monooxygenase where NH_4^+-N is oxidized to hydroxylamine (NH_2OH) with the reduction of one of the atoms of O_2. The hydroxylamine is then oxidized to NO_2^--N by the hydroxylamine oxidoreductase.

The second step in the process of nitrification is aerobic nitrite oxidation to nitrate and is mediated by aerobe chemolithotrophic bacteria, known as nitrite-oxidizing bacteria (NOB), which can also use organic compounds in addition to nitrite, for generation of energy for their growth. In contrast with the AOB, only one species of NOB is found in soil and freshwater and it is the bacteria of genus *Nitrobacter* that belong to the family *Nitrobacteriaceae*, which is one of the families of the order *Pseudomonadales* (Austin *et al.,* 2003). According to these authors, this second step is catalysed by nitrite oxidoreductase without detectable intermediate (Tallec *et al.*, 2006). The reactions for these two sequence steps are the following:

$$2\ NH_4^+ + 3\ O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O + \text{energy}$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- + \text{energy}$$

Nitrite (NO_2^--N) is chemically unstable and usually is found in low concentrations in wetland systems, while nitrate (NO_3^--N) is the oxidized form stable and can be remained untransformed in the various energy-consuming biological nitrogen transformations that occur within wetland systems (Kadlec & Knight, 1996). According to Kadlec & Wallace (2009) when, in effluent from wetland, occurring detectable levels of nitrite must be indicating an incomplete nitrification process.

As shown by the above reaction equations, the process of nitrification requires dissolved oxygen (DO) availability, which is used as electron acceptor, although there are some authors who indicate that nitrifying bacteria are able to tolerate low oxygen concentrations, about to 0.3 $mg.L^{-1}$ of DO (Vymazal, 2007). However, other studies have reported that nitrification is not observed if DO levels is lower than 0.5 $mg.L^{-1}$, and some even show that it is essential an oxygen concentration between 1-2 $mg.L^{-1}$ to not retard nitrification process (Paredes *et al*., 2007).

Stoichiometrically nitrifiers need 4.57 $gO_2.g^{-1}$ of ammonium nitrogen oxidized, which also explain part of the potential depletion of oxygen in receiving water resources where are discharged wastewater, although this depletion may also be due to the oxidation of organic matter presenting in wastewaters (Kadlec & Wallace, 2009). On the other hand, this requirement into oxygen can be compromised by mineralization of influent organic matter, which contributes to the depletion of DO within the HSSF-CWs and hence contributes to slow the process of nitrification. In fact, Lee *et al.* (2009) observed that an increased in BOD levels contribute to decrease nitrification rates in wetland and this it is attributed to the competition for the available DO between nitrifying communities and aerobic heterotrophies microorganisms responsible by organic matter decomposition, which have a relative fast growing compared to the growing of nitrifying bacteria.

So, the availability of DO is considered the most limiting factor of the nitrification process in HSSF-CWs. As we have seen, within HSSF-CWs strong gradients of potential redox occur that are related to the presence of wetland plants and so, they can play an important role in nitrogen removal since promoting the formation of aerobic microsites adjacent to the root system and creating a redox gradient from +500 mV near the root surface to -250 mV at a distance of 1-20 mm from the root surface where ammonia will be oxidized by nitrifying bacteria and so stimulate nitrogen removal in HSSF-CWs (Wiessner *et al*., 2005; Brisson & Chazarenc, 2009). Bezbaruah & Zhang (2004) in experimental HSSF-CWs found that the redox potential at the surface of lateral roots of *Scirpus validus* was higher than that was observed in the bulk water and they attributed this difference to the presence of oxygen released by the roots plant.

Tanner (2001) in a review paper on several studies in which planted and unplanted HSSF-CWs were compared, also, have concluded that although plants only marginally increase the rate of elimination of organic matter, clearly increase the rate of ammonium removal. Dan *et al.* (2011) and Abou-Elela *et al*. (2013) in studies on which they compared systems with and without plants also found that the higher nitrogen removal rates occurred in beds with plants, highlighting the important role of plants in improving the removal rates of this pollutant.

As we said before, water depth affects the redox conditions and is therefore also expected that affect the removal efficiency of nitrogen compounds in HSSF wetland systems. García *et al.* (2005) in a pilot HSSF system observed that a lower water depth contributed to a higher redox potential value and consequently they found that shallower system were more efficient in removing ammonium compounds. The same results was reported by Headley *et al.* (2005) when they doubling the water depth of a HSSF-CWs.

Another way for increasing NH_4^+-N removal is to increase oxygen concentration in CWs through artificial aeration. Nivala *et al.* (2007) reported a percentage of ammonia removal greater than 90% throughout every season using an aerated subsurface flow wetland to treat landfill leachate, despite they also have been observed the lowest ammonia reductions during the winter months but still exceeded 90% removal. The same authors, observed ammonia reductions ranging between 14-40% when aeration was not provided. Also Kinsley *et al.* (2002) in studies at laboratory scale have shown that supplied aeration can have a tenfold increase in nitrification rates. Generally, batch and intermittent feeding seems provide a more oxidized conditions than continuous operation and so promote a higher ammonium removal rate, since nitrification constitute an essential step in removal of this constituent in HSSF-CWs (Kadlec & Wallace, 2009). Stein *et al.,* (2003) observed an ammonium removal efficiency of 57% in batch-operated experimental systems, in comparison to 42% in continuous systems.

Beyond the availability of DO within the HSSF-CWs, nitrification is also influenced by several environmental conditions such as temperature, pH, and alkalinity of the wastewater, HRT and concentrations of NH_4^+-N. The optimum temperature ranges from 30 to 40 ºC, and the minimum temperatures for growth *Nitrosomonas* and *Nitrobacter* are 5 and 4 ºC, respectively (Cooper *et al.,* 1996). Some studies have been demonstrated that the removal rates of NH_4^+-N and TN in winter are lowered by 12% to 40% and 12 to 27%, respectively when compared to the removals occurred in summer (Song *et al*., 2006; Taylor *et al.*, 2011). In fact, according to Xi *et al.* (2003) when temperature falls below 10 ºC the rate of ammonium oxidation seems to be considerably inhibited.

The optimum pH values for nitrification process may vary from 6.6 to 8.0 (Vymazal, 2007). However, along the process for each NH_4^+-N molecule $2H^+$ ions are produced and thus it is consumed a large amount of alkalinity, about 7.07 $mgCaCO_3\,mg^{-1}$ of NH_4^+-N oxidized (Ahn, 2006), which may lead to acidification of the medium, especially in wastewater with low alkalinity and/or high ammonia concentrations and so, liming may have to be added in order to preventing this decreased of pH (According to Ahn (2006) it is recommended values of alkalinity greater than 50 mg.L^{-1} as $CaCO_3$).

According to Lee *et al.* (2009) nitrogen removal requires longer HRT compared with those which are normally referred to as being needed to remove organic matter. Nitrification seems to be a process that requires long HRT due to the slow growth rates that characterize the nitrifying bacteria and so, unsatisfactory hydraulic conductivity conditions can also result in lower nitrogen removal rates, as can happen during periods of intense rainfall or due to illegal discharges (Albuquerque *et al.,* 2009a; Trang *et al*., 2010). Huang *et al.* (2000) reported that NH_4^+-N and TN concentrations in treated effluent decreased with increasing HRT. Also Toet *et al.* (2003) observed a higher nitrogen removal rate in CWs with a HRT of 0.8 days comparing with the results obtained in systems with 0.3 days of HRT, while Avila *et al.* (2014) in hybrid CWs systems also observed that the removal efficiency for the most compounds decreased as the HLR increased.

Some studies also have showed a strong positive correlation between NH_4^+-N loading and ammonia removal rate (Butterworth, 2014). Platzer (1998) cit. by Platzer (2000) reported that in HSSF-CWs nitrification could be observed since the bed area is sufficiently larger in order to achieve that the maximum total nitrogen load not exceed 73 $gT_KN.m^{-2}.year^{-1}$. However, this loading rate is very low and according to Vymazal (2005) nitrification may be difficult to achieve for HSSF wetland systems that are designed for BOD and TSS removal based on the rule of thumb of 5 m^2/p.e., as we have previously mentioned regarding the removal of organic matter.

In CWs, the denitrification process seems to contribute to the removal of 60 to 70% of the total nitrogen (Vymazal, 2007; Wu *et al*., 2014) due to the environmental characteristics that seems to offer suitable conditions for denitrification because in them tend to prevail anoxic and anaerobic conditions (redox potential < +300 mV) and also there are usually a source of carbon for denitrification through exudates release by roots of plants (Mayo & Bigambo, 2005; Wu *et al.,* 2014).

So, after diffusion in anoxic/anaerobic zone of the wetland, the oxidize forms of nitrogen will serve as an electron acceptor that is used by a very large family of heterotrophic facultative anaerobic bacteria that also needed organic carbon as a source of energy (electron donor) (Corstanje *et al*., 2006). It is found that for denitrification 1 g NO_3^--N to N_2 is needed an organic matter equivalent to 2.86 rates gBOD (or 3.02 g of COD) per gram of NO_3^--N (Paredes *et al*., 2007; Meng *et al*., 2014). Complete denitrification to N_2 seems to require higher carbon/nitrogen ratios (Hunt *et al*., 2006). According to Ye & Li (2009) a C: N ratio (measured as mg BOD: mg NOx-N) of less than 2.3 would limit denitrification rates.

The carbon source for denitrification can come from the carbonaceous BOD present in the influent enter in systems but if influent is already low in BOD, the carbon source may be insufficient for bacterial growth and so, the denitrification cannot proceed as efficiently as would be desirable. However, the exudates released by root system of plants or by the decaying plant detritus can also build a source of organic matter (Vymazal, 2007). Brix & Headly (2007) estimated that roots of *Phragmites australis* release of dissolved organic matter at a rate of about 0.25 $t.ha^{-1}year^{-1}$, which according to to them could be important for stimulated the growth of heterotrophies microorganisms responsible by denitrification.

Denitrification is a biochemical process (Figure 2.11) in which nitrogenous oxides (NO_2^--N and NO_3^--N) are converted into nitrogen gas (N_2), with nitric oxide (NO) and nitrous oxide (N_2O) as intermediates. During this process, nitrate is firstly reduced to nitrite by nitrate reductase and then NO_2^--N is reduced to N_2O by nitrite reductase and finally N_2O is reduced to molecular nitrogen (N_2) by nitrous oxide reductase. In this process the enzyme nitrate reductase allows facultative bacteria to utilize oxygen atoms bound in nitrate and nitrite molecules as final electron acceptors during oxidation of organic carbon. Among the species that are capable of denitrification is the *Pseudomonas* spp., but also others genera such *as Alcaligenes* spp., *Flavobacterium* spp. and *Thiobacillus* spp. (Garcia *et al*., 2010). The reactions that occur at the denitrification process pathway can be represented by the following equation, where CH_2O represents the biodegradable organic matter (Wallace & Knight, 2006):

$$5(CH_2O) + 4NO_3^- \rightarrow H_2CO_3 + 4HCO_3^- + 2N_2O\ (g) + 2N_2\ (g) + H_2O$$

According to Sedlak (1991) cit. by Kadlec & Wallace (2009) for practical purposes, denitrification can be ignored when DO concentrations are greater than 1 $mg.L^{-1}$ and an optimum denitrification rate seems to require a relatively low reducing environment (under a redox potential from +350 to +100 mV) (Wiessner *et al.*, 2005; Vymazal, 2007).

The last step of denitrification, i.e., the conversion of N_2O to N_2, is very sensitive to oxygen and redox status, and disturbance of this step results in incomplete denitrification and N_2O emissions (García-Lledó *et al.* 2011; Mander *et al*., 2014). This gas is a potent greenhouse gas which has a global warming potential about 300 times greater than that of CO_2 and has a lifetime of approximately 120 years in the atmosphere (Mander *et al*., 2014).

In addition to redox potential and organic carbon source, others environmental factors are known to influence denitrification rates and consequently the composition of gaseous end-products in the denitrification process (Vymazal *et al*., 1998). In the case of low concentration of biodegradable organic carbon seems to contribute for increase N_2O emission during denitrification while high C/N ratios seems to stimulate complete denitrification ending up with N_2 (Hunt *et al*., 2006). Also nitrite and nitrate concentrations are often considered to be responsible for N_2O production (Kampschreur *et al*., 2009). Very low nitrite concentration (< 0.5 $mg.L^{-1}$) produce dinitrogen as the dominant product, whereas higher nitrite concentrations (> 2 $mg.L^{-1}$) tend to produce as the predominant end product nitrous oxide, probably due to the inhibition of the N_2O reductase by high concentrations of NO_2^--N (Mander *et al*., 2014).

Denitrification is also dependent of temperature and although the temperature range for denitrifies is fairly broad and seems only proceeds at very low rates if temperature is below 5ºC, the process tend to increase with increasing temperature and activity has been measured in temperatures up to 60-75°C (Toet *et al*., 2003). However, it has been suggested that the optimum temperature for denitrification is between 20 to 25 ºC, despite there are conflicting reports as to minimum temperatures which may allow some denitrifying activity (Spieles & Mitsch, 2000; Vymazal & Kröpfelová, 2008).

Substrate pH seems to be one of the most important factors influencing denitrifiers community composition (Philippot *et al*., 2007), which can be an important driver of denitrification activity and N_2O production (Mander *et al.*, 2014). In general, the denitrification rate increases with increasing pH values (up to the optimum pH) while, in contrast, the N_2O/ (N_2O+N_2) ratio decreases (Garcia *et al*., 2010). According to Vymazal (2007), optimal pH is 6-8 and the activity is low at pH 5 and absent in pH below 4. Contrary to the process of nitrification, during denitrification alkalinity is formed which this can result in rises in pH value.

In CWs systems, the nitrogen chemistry seems governed by classical nitrogen pathways, but along the time it has been reported other NH_4^+-N removal mechanisms such as ammonia volatilization, adsorption, plant uptake and others like Anammox pathway, an autotrophic process (Paredes *et al*., 2007; Joss *et al.*, 2011). This later process seems quite interesting because it is able to directly convert NH_4^+-N to N_2 without the release of the aforementioned gases (NO and N_2O).

At present, the studies available about the role of Anammox as a potential removal route for ammonia in HSSF-CWs are still scarce (Coban *et al*., 2015). In this process, anaerobic conversion of NH_4^+-N to N_2 occur mediated by a group of *Planctomycete* bacteria, which shows that ammonia can be directly reduced to N_2 using NO_2^--N as an electron donor without carbon source and N_2O production, as showed the following stoichiometry reaction (Van de Graaf *et al*., 1996; Strous & Jetten, 2004; Hunt *et al*., 2006):

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13\ H^+ \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.066\ CH_2O_{0.5}\ N_{0.15} + 2.03\ H_2O$$

According to Anammox stoichiometry 1.9 gO_2 is required for 1.0 g of NH_4^+-N, which includes the oxygen needed to convert ammonia to nitrite (Zhang *et al.,* 2010) and that is much less than the oxygen requirement for standard nitrification/denitrification. The Anammox reactions need NH_4^+-N/NO_2^--N in a ratio of 1:1.3 (Bagchi *et al.*, 2012). However, while these organisms have been found in many natural environments including conventional wastewater treatment systems and grown in sufficient quantities for scale-up reactors, it is still quite unknown the extent of these reactions in the nitrogen cycle within HSSF-CWs (Joss *et al.*, 2011).

So, research is needed to better understand how the microbes and the ammonia oxidizing reactions compete in the ecology of varied wetland systems, despite the opinion of Zhu *et al.* (2011) and Tao & Wang (2009) that is to maintain and develop Anammox bacteria in CWs several factors that including pH, alkalinity, dissolved oxygen concentration, ammonia concentration (high ammonia loading seems to stimulates Anammox bacteria) and nitrite concentrations should be considered because the growth of these bacteria will be dependent on the availability of nitrite.

Assimilation by the living organisms can also contribute to TN removal in HSSF-CWs (Vymazal, 2010; Saeed & Sun, 2012), verifying that plants prefer to assimilate nitrogen in form of ammonium instead of nitrate because the NH_4^+-N is more reduce energetically (Kadlec & Knight, 1996). However, according to Brix (1994), in temperate regions and when these systems are used for secondary treatment systems, the most common use in Portugal, the amount of nutrients that can be removed by harvesting are generally insignificant compared to the loading enter in the system by wastewater. Thus, this may explain the fact that in these regions is uncommon proceed to the cutting of the plants. However, in tropical regions where seasonal translocations are minimal and multiple harvests are possible, it seems that emergent plants could play a significant removal route of nitrogen removal especially for lightly loaded systems. However, if HSSF-CWs are used for polishing purposes, the removal of nutrients through harvesting may play more important role.

Generally, in the literature is referred that the uptake capacity of emergent macrophytes and thus the amount that can be removed if the biomass is harvested is roughly in the range 200-2500 kg $N.ha^{-1}.year^{-1}$ and it has been reported that under optimum conditions the amount of nitrogen removed with the biomass can achieve 10-16% of the total removed nitrogen in wetland systems (Brix, 1994; Vymazal *et al.,* 1998; Mitsch & Gosselink, 2000).

In the literature there are several examples that compares the removal efficiency of unplanted and planted systems and the results, although controversial, show that generally the presence of the plant tend to increase the efficiency of removal of nitrogen compounds (Chung *et al.,* 2008; Debing *et al,* 2010). Coban *et al* (2015) use the stable isotope composition of multiple N species in order to compare pathways of NH_4^+-N removal in a study with planted and unplanted HSSF-CWs at Leipzig, Germany and they concluded that plant presence was an important factor in NH_4^+-N removal efficiency, even though also have verified that most of the NH_4^+-N was removed via nitrification-denitrification processes in planted CWs throughout the year. In fact, they observed that unplanted system had statistically significantly lower NH_4^+-N removal in comparison with planted system and they attributed this fact to the role of plant roots and rhizomes as surface area for attachment and successful growth of microorganisms.

In addition to biological nitrogen elimination, NH_4^+-N ion can be temporarily removed from the water column by adsortion as an exchangeable ion in the surface of media particles (Figure 2.11), although can be released easily when water chemistry conditions change (Mitsch & Gosselink, 2000). In fact, at a given NH_4^+-N concentration in the water column, a fixed amount of ammonia is adsorbed to and saturates the available attachment sites. When the NH_4^+-N concentration in the water column is reduced, (*e.g.* as a result of nitrification), some ammonia will be desorbed to reach again the equilibrium with the new concentration. However, the adsorption capacity of the porous media (commonly coarse-grained material) usually used in HSSF-CWs is low and therefore nitrogen removal through this via is very limited in these systems (Kadlec & Knight, 1996; Vymazal, 2005).

Ammonia volatilization is another pathway for nitrogen loss to the atmosphere from wetlands systems and is a physicochemical process where ammonium nitrogen becomes volatile as ammonia non-ionized form (NH_3) (Table 2.5; Figure 2.11). This conversion is dependent on pH and temperature and it was observed that for lower pH and temperatures levels, this conversion is insignificant (Kadlec & Wallace, 2009). A wastewater with 25 ºC of temperature and a pH of 7.0, NH_3 seems to represent only 0.6% of the total ammonia present, while for a pH equal to 9.5 and a temperature of 30 ºC the amounts of NH_3 increases to 72% and the ratio ammonia and ammonium ion is 1:1, which means that the losses via volatilization are significant (Vymazal, 2007; Kadlec & Wallace, 2009). In the case of HSSF, the volatilization may be less important as a nitrogen removal process because these systems do not have a free water surface and also because they have an insignificant algal photosynthesis and so did not creates a higher pH values during the day which could be favorable for ammonia loss.

Recently, some innovations have been tested in order to contribute to enhance the nitrification process in HSSF-CWs and thereby contribute to increasing the overall nitrogen removal by these systems (Tee *et al*, 2012; Pearce, 2013). Some studies tested wet-dry cycling, which is a new method for introducing oxygen into the substrate. In tidal flow CWs, wastewater is not fed continuously, but is controlled by a flood-drain cycle. During the cycle, oxygen enters the substrate during the drain period, thereby providing oxygen for nitrification (Wu *et al.* 2014). Another improvements is the use of hybrid system, such as for example using a VSSF followed by a HSSF-CW system, to ensure better conditions for nitrification in VSSF, more aerobic, and then denitrification in HSSF-CWs where oxygen is more limited (Vymazal, 2007). However, as we have seen, denitrification requires sufficient biodegradable organic content to pursue favorably (Metcalf & Eddy, 2003) and in VSSF-CWs 90% of influent organic matter in domestic wastewater can be removed, therefore, at this type of arrangement, the organic matter content present in HSSF may not be enough to ensure an efficient denitrification process.

For this reason, some studies tested a reverse arrangement, which seems to ensure adequate biodegradable organic material for denitrification, but appears to be necessary to recycle the effluent VSSF system to the head of HSSF though it seems necessary to recirculation of the VSSF system effluent to the head of HSSF in order to achieving greater TN removal rates. Cooper *et al*. (2010) in a hybrid system (HSSF-VSSF) when testing two recirculation:influent ratio (1:1 and 1:4), observed a significant relationship between this ratio and TN removal and their results showed that TN removal was 50% and 80%, respectively. Thus it seems that increasing the recirculation ratio may significantly increase nitrogen removal, although this will also contribute to increase the surface area required for the treatment.

2.4.4. Phosphorus removal

Municipal and industrial wastewaters discharges as well as agricultural runoffs seem to be the main sources of the phosphorus (P) loads that flows into water resources, and their P contents derived from human and animal dejections, food waste, P-based detergents and P-based fertilizers. These P loads usually result in the enrichment in P of the receiving water bodies and as P is an essential nutrient for biomass growth, like nitrogen, can cause an uncommon growth of algae and aquatic plants resulting in eutrophication of water bodies with significant negative impacts such as anoxia and dead bottoms, which can result in severe ecological and economic consequences.

Phosphorus in domestic wastewater may be present as organic or inorganic fraction in particulate (PP) and dissolved forms (DP). The sum of all forms of phosphorus is the total phosphorus (TP), which typical concentrations in municipal wastewaters range from 5 to 15 mg TP.L^{-1}, of which1-5 mg.L^{-1} is particulate organic P and 4-10 mg. L^{-1} is soluble inorganic P (polyphosphates, and predominantly orthophosphates) (Metcalf & Eddy, 2003). According the same authors, inorganic phosphorus is the primary form in wastewater representing over 75% of total phosphorus.

Dissolved inorganic P is considered bioavailable and is composed mainly by orthophosphate (PO_4^{3-}, HPO_4^{2-}, and $H_2PO_4^{-}$), although it may include readily hydrolysable organic phosphorus, whereas organic particulate P forms generally must undergo transformations to inorganic forms to be considered bioavailable. Biological decomposition of organic phosphorus results in the conversion to orthophosphates forms. The chemical equilibria between all phosphate forms depend on the pH of the solution, verifying that $H_2PO_4^-$ is the primary form when pH ranges from 2 to 7, HPO_4^{2-} dominates when pH is between 7 and 12, and finally PO_4^{3-} becomes the main form for values of pH >12 (Kadlec & Wallace, 2009). Polyphosphates can also be hydrolyzed to orthophosphates and its presence is mainly related to the use of synthetic detergents (Metcalf & Eddy, 2003).

Nowadays the reduction of P loads into water bodies is a priority in terms of management and preservation of water resources, with stricter controls on wastewater discharge, especially in areas that present a high risk of eutrophication and that are classified as sensitive areas. In Portugal the quality requirements for discharges from municipal wastewater is established by Decree-law 152/97 of 19 June, which implemented the Directive 91/271/EEC. This Decree-law only required to meet specific P values when municipal wastewater is discharge occur in a water body identified as particular sensitive area that are subject to eutrophication and the standards required is 2 mg TP. L^{-1} for 10,000 – 100,000 population equivalent and 1 mg TP. L^{-1} for WWTPs treating more than 100,000 PE.

For small WWTPs, defined as treatment facilities receiving wastewater equivalent to less than 2,000 PE, the current legislation only defines the need of an appropriate wastewater treatment in order to minimize negative impacts of its discharge into natural environment receiving bodies.

However, the limit value for Phosphorus in small WWTPs can also be covered by other relevant EU regulations such as the EU Water Framework Directive (2000/60/EC) that demand improvements in surface water quality and which generally also require a reduction in nitrogen (N) and phosphorus (P) from wastewaters for achieve “good chemical and ecological status” in water resources by the year 2015.

The removal of phosphorus in HSSF-CWs, such as nitrogen, has proved to be variable and lower than for organic matter or TSS, probably due to the complex dynamics interactions of numerous parameters such as redox potential, pH, Fe, Al and Ca minerals in the substrate media, HRT, HLR, P loading rate and plants each of which may cause changes in P supply, uptake or release internally in wetland system (Cui *et al*., 2008). According to Vymazal (2002) and based on the efficiency of different HSSF-CWs at several European countries, means TP removals ranged between 26.7% and 61.4%. However, the removal efficiency is highly variable, and sometimes releases of P have been observed (Babatunde *et al*., 2010).

Figure 2.12 shows the major and complex P transformations and inter-conversion of all forms of phosphorus in wetlands, where DIP means dissolved inorganic P, DOP corresponds to the P combined with dissolved organic matter which is generally readily hydrolyzed to inorganic P so, together with DIP are sometimes referred as soluble reactive P (SRP). P associated with suspended particles is termed as particulate organic P (POP) and particulate inorganic P (PIP), while IP means inorganic P.

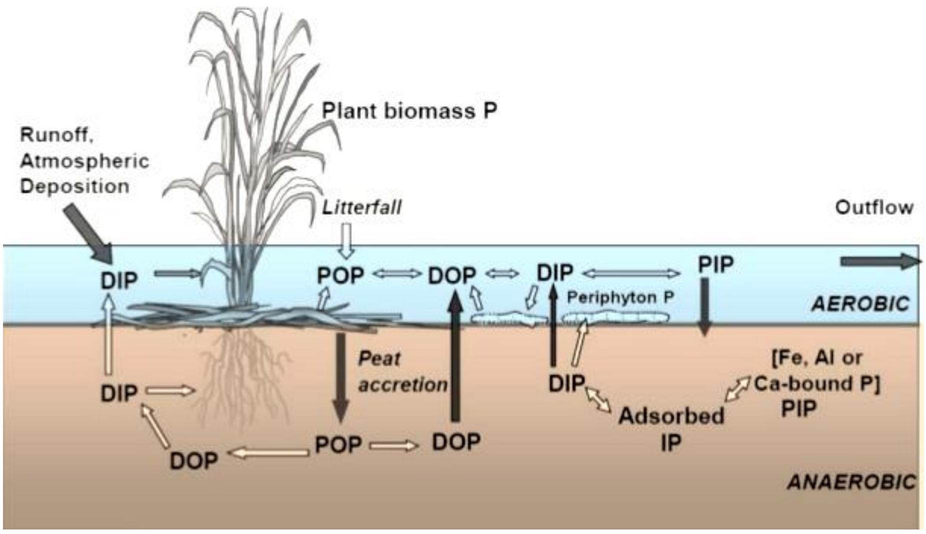

Figure 2.12 Schematic diagram showing the transformations of phosphorus in wetland system (Reddy & DeLaune, 2008)

Adsorption, the process by which soluble inorganic P is moved from pore water onto soil mineral surfaces through ionic exchange mechanisms is considered the main mechanism that control long-term P retention in HSSF-CWs due to the usually low solubility of some phosphate minerals compounds (Vohla *et al.,* 2011). According to Anderson & Magdoff (2005) sorption mechanism is limited to soluble inorganic P and some reactive soluble organic P and the net direction of flux is controlled by the pore water P concentration and the affinity of the media particles to P ions (Reddy & DeLaune, 2008). An additional pathway for P removal in HSSF-CWs it is the chemical precipitation reactions, during which phosphates react with some metallic ions and form insoluble compounds.

Sorption and precipitations reactions are controlled by physical and chemical characteristics of the material substrate as we have seen in section 2.3.1 (e.g. size and content and presence the fraction of minerals such as Fe, Al, Ca and Mg) and by some physic-chemical environmental conditions (e.g. pH, redox potential, dissolved ions) and hydraulic parameters (*e.g*. HLR, HRT) (Anderson & Magdoff, 2005; Cui *et al*., 2008). According to Reddy & DeLaune (2008), Fe, Al and Ca oxides enhanced the P adsorption capacity of the filter media and finer textured media tend to have greater ability to adsorb P than coarser textured like gravel beds.

The phosphorus can be adsorpted to organic and mineral surfaces of media material and occur until the exchange sites are saturated and the time period to saturate can be long for the media particles that have high sorption capacity and vice versa (Seo *et al*., 2005). Precipitation reactions with Fe and Al that under oxidized conditions can be form hydrated ferric and aluminium oxides which can contribute for precipitation of P as ferric phosphate and aluminium phosphate. Substrate media rich in calcareous can also result in formation of various precipitates of P such as calcium phosphate, dicalcium phosphate and beta-calcium phosphate (Reddy & DeLaune, 2008). It is difficult to quantify precipitation separately from adsorption, since the most of the times the precipitates occurs on the surfaces of media particles (Anderson & Magdoff, 2005; Vohla *et al*., 2011).

For this reason, several studies have tried to assay the use of alternative materials potentially with higher phosphorus adsorption capacity and/or rich in iron, aluminum and calcium, in order to increase the precipitation and/or adsorption of this element within the bed. Some of these media are natural materials (e.g. limestone, opoka, wollastonite, dolomite, marble, lime gravel, zeolite), industrial by-products (e.g. blast furnace slag, steel furnace, coal fly ashes, Ochre, cement clinker) and also some man-made materials (e.g. filtralite®, Lightweight aggregates (LWA) or light-expanded clay aggregates (LECA) (Àdàm *et al*., 2005; Xu *et al*., 2006, Chazarenc *et al*. 2007; Albuquerque *et al*., 2009b; Ballantine & Tanner, 2010).

Arias *et al.* (2001) have study 13 Danish sands and found that their P removal capacities varies among systems due to the different origin and were influenced more strongly by Ca content than by Fe and Al content and they attributed this fact to the character slightly alkaline of the municipal wastewaters. Xu *et al*. (2006) also evaluated different sands that show a varying P sorption capacity from 0.13 to 0.29 $g.kg^{-1}$, which they attributed to the fact that in sand substrates, P is bound to the medium mainly as a consequence of adsorption and precipitation reactions with Ca, Al and Fe and sands do not all have the same composition and so, the same adsorption/precipitation capacity.

In laboratory tests comparing the P-sorption capacities of limestone, wollastonite, zeolite, LECA and sand, Yin *et al.* (2006) found that limestone have the best P sorption characteristics for use in subsurface-flow constructed wetlands, while in field studies, Comeau *et al*. (2001) reported 92% P removal from trout farm effluents in HSSF-CWs with crushed limestone media. Vohla *et al.* (2005) found a retention capacity of up to 3.2 $gP.kg^{-1}$ for LECA, while Öövel *et al.* (2007) in a HSSF filled with LECA observed a TP removal of 89%. Korkusuz *et al*. (2005) compared P removal in gravel wetland bed and blast furnace slag and concluded the later media showed far better P sorption capacities. Drizo *et al*. (2006) also reported good results with steel slag.

Rosolen (2000) observed that blast furnace slag and cement clinker were the best media for phosphorus removal from amongst the 21 media who were tested. Cement clinker is a silica based medium consisting primarily of lime (CaO) and is produced during the production of cement. It is porous in nature and also contains aluminium and iron oxides and some trace minerals. Chen *et al.* (2006) show that coal fly ashes can be contributed for retention P at rate up to 42.6 $gP.kg^{-1}$. Heal *et al*. (2005) in laboratory experiments study the use of ochre (rich in ferric oxide) from acid mine drainage treatment and they concluded that is a promising material as substrate media for P removal in HSSF-CWs, having gotten a maximum P adsorption capacity of 26 $g.kg^{-1}$ of the material. However, in field experiments the values obtained for adsorbed P ranged from 16 to 18 $g.kg^{-1}$ of the ochre used as bed media.

Westholm (2006) published an extensive review of P sorption substrate and conclude that wollastonite, slag material and LECA are the most suitable materials for P removal. Vohla *et al.* (2011) more recently also published a review on the same subject and has conclude that, although the major investigations have been performed in the batch experiments, the results suggest that the P retention capacity of most filter materials significantly decreases after a 5-year period of application. They also observed that the highest P removal capacities were reported for various by-products (up to 420 $gP.kg^{-1}$ for some furnace slags), followed by some natural materials like heated opoka that presented a maximum P sorption capacity of 40 $gP.kg^{-1}$ and finally man-made filter media with a maximum of 12 $gP.kg^{-1}$ for Filtralite. Ballantine & Tanner (2010) offered an ample list of soil amendments and filter media that could be used to improve P removal efficiencies in constructed treatment wetlands, having Calheiros *et al*. (2009) suggested that substrate and amendment choices should also consider their ability to support and propagate macrophytes necessary for nutrient abatement.

In addition, a suitable filter material for P removal should also have a size to be small enough to provide a high surface area to ensure a high P sorption potential, since the maximum P adsorption capacity of a filter medium generally increases as the filter medium size decreases due to the consequent increase in their specific surface adsorption (Seo *et al.*, 2005; Vohla *et al.*, 2011). Akratos & Tsihrintzis (2007) tested three different porous media in pilot-scale HSSF systems and they observed that greater removal efficiency of PO_4^{3-}-P has occurred for fine gravel substrate media (D50 = 6 mm; range 0.25-16.0 mm) (88.6%), followed by medium gravel (D50 = 15 mm; range 4-25 mm) (66.9%) and finally coarse gravel (D50 = 90 mm; range 30-180 mm) (57.3%).

Garcia *et al*. (2005) also examined the effect of the media size on P removal and they observed that P concentration in effluent was lower in bed filled with finer gravel. However, the fine texture of the particle materials can cause problems in bed permeability due to its low hydraulic conductivity and thus can lead to overland flow and incomplete contact of substrates with sewage in CW systems, so it is also crucial, to achieve a compromise between a high specific surface and simultaneously an appropriate permeability of the bed (Kadlec & Knight 1996; Cui *et al.*, 2008). Therefore, an ideal substrate should possess both a high P sorption capacity and a suitable percolation rate.

Regardless of the medium filtrate used, for an effective adsorption and precipitation of phosphorus in HSSF-CWs systems, the wastewater must be in contact with the filter media, which is a characteristic of these systems (Vohla *et al*., 2011). Generally, longer HRT and low HLR are considered to increase P removal because under these conditions there is a greater possibility of contact between the wastewater and the surface of substrate particles and a higher diffusion rate of wastewater through the matrix media and so potential higher access to sorption sites (Vohla *et al*., 2011). Chung *et al*. (2008) showed P removal in vegetated HSSF-CWs of 67% and 52%, at 10 and 5 d HRTs, respectively, while Akratos & Tsihrintzis (2007) showed that HRT also play a significant role on P removal efficiency which tends to increase with increasing HRT.

Afrous *et al.* (2010), also study the effects of retention time (1.5, 3.4 and 7 days) on the P removal efficiency and showed that maximum removal efficiency was observed for 7 days retention time with amount of 56.7% and 45.6% respectively for TP and $PO4^{3-}$-P. Garcia *et al.* (2005) and Drizo *et al*. (2006) also observed that higher HLRs have produced consistently higher P effluent concentrations. However, some authors did not found any effect from HLR on P removal (Toet *et al*., 2003).

pH seems to be one factor that influences the adsorption and precipitation reactions that occur, and it was found that for pH values greater than 6, the reactions are a combination of physical adsorption to iron and aluminium oxides and precipitation as insoluble calcium phosphates, which is the dominant transformation at pH greater than 7.0, while at lower pH levels, P is fixed mainly by precipitation as iron and aluminium phosphates (Vohla *et al.,* 2011). Al and Fe oxides can occur as amorphous (predominantly found in anaerobic flooded media, typical of the HSSF-CWs) and/or as crystalline structures (predominantly found in aerobic environments) and the first structures seems to exhibit greater reactive surface areas than crystalline forms for P sorption (Drizo *et al.*, 2006).

Redox potential can also affect the solubility of Fe-ligand minerals compounds and thus the availability of P in wetland media substrate. Under oxidizing conditions, Fe exists in the form of ferric iron (Fe^{3+}), whereas for reducing conditions, this ion is reduced to ferrous iron (Fe^{2+}). Thus, P adsorbed onto Fe-containing minerals could become mobile when Fe^{3+} is reduced to Fe^{2+}. Redox potential below +250 mV will cause the reduction of Fe^{3+} to Fe^{2+}, releasing associated P and so; affect the substrate media ability to retain P (Mitsch & Gosselink, 2000). According the same authors, aluminium seems to be not involved in oxidation-reduction reactions and is only affected by changes in pH. Garcia *et al.* (2005) to study the effect of water depth on P removal in HSSF wetland systems found that shallow system (with a depth of 0.27 m) removed greater quantities of SRP than systems with depth of 0.5m, but only during the first two growing seasons and they attributed these results to the higher redox potentials and oxygen concentrations in shallow beds and the greater degree of contact with plant roots.

In the HSSF systems tend to prevail anaerobic conditions (lower redox potential) due to permanent and continuous waterlogging and taking into account also conventional design and typically filter media used (e.g. gravel and sand) that usually have low capacity for sorption and precipitation reactions due to its small specific surface area and/or usually low levels of Fe and Al, it may be reasonable to assume that these systems do not have a sustainable and high capacity for P removal from wastewater (Korkusuz *et al*., 2005).

In addition to the processes of adsorption/precipitation, others mechanisms (e.g. particulate settling, plants uptake and bacterial immobilization biological uptake) may contribute to phosphorus removal, but they seem to play a secondary role in the removal of phosphorus. According to Matos *et al*. (2010) about of 97% of TP removed is by physical mechanisms, 2.1 % by microbial activity and as little as 0.3% by uptake of phosphorus from plants.

Plants and microorganisms typically utilize directly only SRP, while the others forms of P must be first hydrolysed before they can be taken up biologically. Generally, the authors agree that vegetation has a positive effect on P removal from wastewaters in HSSF-CWs and attribute this effect to the absorption of phosphorus by the roots and its conversion into plant tissues (Hijosa-Valsero *et al*., 2012; Shelef *et al.,* 2013). Phosphorus concentrations in plant tissues are much lower than that of nitrogen and so, the uptake P capacity of macrophytes is relatively lower when compared to nitrogen and with the inflow P loadings rate (Brix, 1994; Vymazal, 2007). Generally, the rate of absorption of P is highest during the beginning of the growing season (in Portugal during the spring season), before maximum growth rate is attained (summer) and depend on the concentration of nutrients within the plant tissue (Shelef *et al.*, 2013). According to Kadlec & Knight (1996), the concentration of P in the leaves of emergent macrophytes range from 0.1 to 0.4% on a dry weight basis.

In a study at pilot-scale to assess the performance of HSSF-CWs under tropical conditions, Mburu *et al*. (2013) obtained a TP mass removal efficiencies ranging between 29.2-42.7% and 30.0-63.3% for unplanted and planted system with *Cyperus papyrus*, while the soluble reactive P in planted system show an efficiency between 17.1-76.9%, whereas in unplanted system there was mainly an increase of phosphates effluent concentrations with removal efficiencies of -35% to 3.7%. This data indicate that plants play a role in the removal of P due the uptake of this nutrient.

Debing *et al.* (2010) also showed that HSSF gravel constructed wetlands removed more P when vegetated and the removal efficiency was about 81% in planted wetlands.

Generally it is considered that minimal phosphorus removal is achieved by plant uptake and to make it permanent the biomass needs to be harvested to prevent release back to the water during the decomposition of plant litter (Vymazal, 2007). Similar to nitrogen, the amount of P removed via harvesting of emergent macrophytes is low, especially when inflow loadings are high, despite could be important in CWs which have low inflow loading (10-20 $gP.m^{-2}.year^{-1}$). Kadlec & Wallace (2009) referred that when plants are harvested the amount of P which can be removed is about 2 - 4.9 $g.m^{-2}.year^{-1}$, which is a very small amount since the primary influent P loading usually is about 150 $gP.m^{-2}.year^{-1}$. Field experiences also suggests that P removal by plants and subsequent harvesting will only account for a small percentage, generally < 5% of the TP removed in HSSF-CWs (Brix, 1994), but Tanner (2001) indicates that plant P uptake is responsible for 6-13% P removal from wastewater applied to the CWs.

Mancilla-Villalobos *et al.* (2013) observed that the concentration of P in plant tissue varies both among species and during the season. They observed that P removal decreased during winter, probably due to the decrease in plant density and also found negative removal rates in early spring which have attributed to the death plants in previous seasons, which may have constituted an additional source of phosphorous within the system due to its mineralization. So, a larger plant growth during late spring and summer seems to improved P removal, while in the colder season it diminished. These results are consistent with what has been observed by other authors (Stottmeister *et al.* 2003; Akratos & Tsihrintzis, 2007; Vymazal, 2007).

Afrous *et al.* (2010) study the removal of TP and PO_4^{3-}-P in experiments conducted in mesocosm scale in Iran, under a climate warm with Mediterranean rainfall regime. In this research, *Phragmites australis* and *Typha latifolia* performance were compared to a control (unplanted) system. In all three systems, influent phosphorus concentration was 10 mg/L. Results showed that concentrations of PO_4^{3-}-P and TP in effluent of flow after 7 day reduced to average 56.7% and 45.6% respectively for three systems. Maximum removal efficiency was observed for system contains *Phragmites australis* by amount of 71.5% and 63.3% for PO_4^{3-}-P and TP concentrations in effluent. However, the contrary was observed by Akratos & Tsihrintzis (2007) who obtained significantly lower efficiencies for systems planted with *Phragmites australis* (43.9%) compared to the *Typha* spp. (66.9%). The same trend was also observed for TP.

2.4.5 Pathogens removal

Domestic wastewaters have in its constitution several species of human enteric organisms which can be found attached to suspended solids or in suspensions in wastewaters that represents a serious public health concern due to the potential transmission of infectious disease via waterborne pathogenic microorganisms, including bacteria, protozoans, helminths, and viruses

(Metcalf & Eddy, 2003). WHO (2006) estimates that diarrhoea alone is responsible for 3.2% of all deaths worldwide and in addition to diarrhoea, that International Organization estimates that each year, 16 million people contact typhoid and over one billion people suffer from intestinal helminth infections. The potential reuse of wastewater introduces a potential risk of infection that may occur through inhalation, ingestion or topical contact with reuse water.

Many of these pathogens in excreta and wastewater can survive in the environment enough time to be transmitted to humans through contact with excreta or wastewater or consumption of contaminated products irrigated with wastewater. Viruses for example can survive 50-120 days, bacteria like salmonella spp. 30-60 days, protozoa up to 180 days and helminths eggs many month up to years (measured in selected environmental media at 20-30°C) (WHO, 2006).

Hence, it is important to use an appropriate wastewater treatment that can minimize the pathogen risks, especially in regions where wastewater is used as a source of irrigation or when wastewater discharges occurs in receiving bodies that are also bathing waters. Several studies on microbial water quality improvement have shown that CWs have the potential to reduce various types of enteric pathogens in wastewaters, especially faecal group, at varying but important degree of efficiency thus contributing to the reduction of risks for public health (Reinoso *et al.,* 2008; Rai *et al*., 2013; LLorens *et al*., 2011).

Within the HSSF bed of macrophytes the pathogenic microorganisms have to compete with the autochthonous organisms and as they require a higher temperature and a rich substrate most of them do not survive. Its removal is achieved by a variety of processes that include physical processes and chemical and biological removal mechanisms that may involve adsorption and oxidation mechanisnms and exposure to biocides excreted by roots of wetland plants. They also can be removed by natural die-off.

Survival of pathogen microorganisms is also dependent upon the presence of others organisms in the water (*e.g*. nematodes, rotifers and protozoans) which may provide competition for limiting nutrient and predation. Puigagut *et al.* (2007) have been found that free-living protozoa are the principal grazing microfauna that can contribute for removal pathogens in CWs. The contribution of each of the above ways is suggested to be a function of wastewater flow rates, nature of the macrophytes and type of the wetland (Mburu *et al*., 2013).

The major studies of microbial pathogens removal in CWs have dedicated on total and faecal coliform removal and they reported removal efficiencies in HSSF-CWs that typically exceed 90%, with significantly higher removal rates reported for vegetated systems (Vymazal, 2005; Reinoso *et al*., 2008) and generally the studies carried out either pilot scale or full scale showed that in these systems can be achieved a linear decay in a logarithmic scale, around 2 to 3 log units of reduction in pathogen indicator organisms (Vacca *et al.*, 2005; Vymazal, 2005; Tanner *et al*., 2012).

Song *et al.,* (2008) reported 99.7% reduction of fecal coliforms in Asia, while Reinoso *et al.* (2008) reported the removals of 96% for *Escherichia coli (E. coli)* and faecal streptococci in Europe systems. Keffala & Ghrabi (2005) and Mburu *et al.* (2013) obtained removals of 90% and 99.9% for fecal coliforms, respectively for Africa systems. Vymazal (2005) reported an average pathogens removal of 92% for HSSF-CWs, based on data from worldwide experience in total of 51 systems evaluated, for fecal coliform. For Portugal, Galvão *et al.* (2009) reported for two full-scale HSSF-CWs an average bacterial reduction rates around 2 log units (logarithmic units/100 mL) for total coliforms, fecal coliforms and for *E. coli,* while for Enterococcus they observed a reduction from 1.2 to 2.1.

However, the fate of microbial pathogens in these systems seems to be also influenced by various factors such as climatic conditions, HLR, HRT, substrate media and the design specifics of the wetland itself (García *et al*., 2010). Generally, removal is enhanced under longer HRT or lower HLR, shallower bed depths and higher warmer water temperatures (Morató *et al*., 2014). Some studies reported that the granulometry of substrate media in CWs can influenced the faecal bacteria removal, with small size granular media to enhance the reduction from 1.0 to 2.0 log units for both faecal coliforms and coliphage (Garcia *et al*., 2010).

Meira *et al. (*2013) in pilot-scale HSSF-CWs vegetated with rice (*Oryzasativa* L.) have study the effect of two different media (sand and gravel) on thermotolerant coliforms and observed that they were reduced more than 6×10^5 CFU.100 mL^{-1} down to less than 2.5×10^4 CFU.100mL^{-1} in system filled with sand being overall removal efficiencies of more than 97% (between 1.58 and 2.64 log units); while system filled with gravel reached slightly lower removal and which ranged between 94.9 and 98.6% (between 1.29 and 1.85 log units). These performances are comparable to that (1.7 log) reported by Gauss (2008) also for a pilot-scale HSSF-CWs filled with gravel at Masaya, Nicaragua. Also, regarding to faecal streptococci vegetated constructed wetlands filled with sand reached better performances (98%) being effluent mean counts less than 3000 CFU.100 mL^{-1}, than those filled with gravel (94%) being mean effluent CFU between 2000 and 6500.100 mL^{-1}.

Morató *et al.* (2014) observed in a study at pilot-scale that the HSSF wetland with a water depth of 0.27 m and a granular media size of 3.5 mm are more effective than those with a water depth of 0.50 m and a granular media size of 10 mm for removal total coliform, *E. coli*, fecal enterococci, and total heterotrophic cell numbers (22 ºC) and they explained these results by the fact that larger fraction of the water volume is in contact with the root system of plants in HSSF wetlands with fine media. They observed that removal in all HSSF wetlands occurs near the inlet zone by a combination of biological and physical mechanisms and the overall microbial removal ratio ranged between 1.4 and 2.9 log-units for total heterotrophic cell numbers, while for total coliform the values obtained ranged from 1.2 to 2.2 log units for total coliforms and from 1.4 to 2.3 log units for *E. coli*. Concerning to the season effect, they observed a significant higher removal rate for bacterial indicators in summer and attributed this fact to the higher temperature.

With regard to seasonal differences, some authors also have reported higher removal performance during the warm season (Karathanasis *et al.*, 2003) whereas others have not found seasonal differences (García *et al.*, 2005, Reinoso *et al.,* 2008). Also Galvão (2009) in the study cited above did not found significant differences for any of the studied microorganisms between summer and winter.

Despite some authors did not found any differences between planted and unplanted systems (Vacca *et al.,* 2005), several studies support the observation that wetlands with vegetation are better than non-vegetated wetlands at removing bacteria from wastewater (Avelar *et al*., 2014). Karathanasis *et al*. (2003) observed that wetlands planted with polycultures perform consistently year-round, with removal rates for fecal coliforms ranging from 98% in the fall to 82% in the winter. Emergent vegetation provides resistance to flow and thereby slows surface water flow and because bacteria usually adsorb to small particles that take longer to settle out of the water column (Kadlec & Knight, 1996), an increased HRT induced by vegetation may favor the sedimentation and thus bacteria removal. In fact, the results of some studies suggest that there is a positive correlation between bacterial removal and HRT. However, Garcia *et al*. (2003) verified that increased in HRT over a period of three days did not widely increase the removal rates or reduce the microbial concentrations in effluent

Martin (2012) compared with the inlet, also obtained average reductions of 1.6 and 0.35 log units at the outlets of the vegetated and non-vegetated wetlands, respectively during the dry season and 1.2 and 0.27 log units during the rainy season. These corresponded to percentage removals ranging from 87 to 95% and from 24 to 71% respectively. These results are also within the range of removals reported for CWs treating domestic wastewater in similar conditions by other authors (Greenway, 2005; Reinoso *et al*., 2008). However, Mburu *et al.* (2013) observed that non-vegetated bed was in the average more efficient than the vegetated wetland in a subsurface horizontal flow system. Kadlec & Wallace (2009) have reviewed data of fecal coliform removal in planted and unplanted HSSF-CWs systems and they concluded that usually the presence of plants has a positive effect on the removal rates.

Frazer-Williams (2010) in a review of the influence of design parameters on the performance of CWs have concluded that coliform removal in HSSF systems was very weakly correlated to HLR and influent loading, which may explain that is usually referred a high removal rate for indicator microorganisms, despite variations in both influent load and HLR. Similar results were obtained by Reinoso *et al*. (2008).

Little research has been carried out into the effect of CWs on the removal of some specific bacterial pathogens; however it seems that bacterial pathogens like Salmonella and Shigella are removed to a lesser extent than fecal indicators. The same occur with viral indicators of wastewaters origins (bacteriophage), despite Thurston *et al.* (2001) reported that the average removal rates for these microorganisms are slightly below 95% for HSSF-CWs. For pathogenic protozoans (*e.g. Giardia* and *Cryptosporidium*) and parasites such as helminthes, there are also very little data about its removal. Some authors found that HSSF-CWs can be remove substantial proportions of these organisms, but also at lower rate than the common values referred for total and faecal coliforms (Stott *et al.*, 2001). Thurston *et al*. (2001) observed an average removal rate of 88% and 65% for *Giardia cysts* and *Cryptosporidium oocysts*, respectively, when a secondary effluent pass through a HSSF-CWs. Kadlec & Wallace (2009) based on data of 44 CWs treatment systems, reported an average log units reduction of 2.53 for *E. coli*, 2.04 for *Clostridium perfringes*, 2.47 for *Salmonella*, 2.00 for *Shigella* and 2.43 for Streptococci fecal.

Despite, the percentage reductions of faecal coliforms and *enterococci* are very promising, it is important to realize that many times the concentrations of these microorganisms in the final effluent remains very high. Usually, for total and faecal coliforms the outflow concentrations are in the range of 10^2 to 10^5 CFU/100mL while for faecal streptococci the range is between 10^2 and 10^4 CFU/100mL (Vymazal, 2005). So, the use of HSSF-CWs as a single treatment process for wastewaters that contain high levels of those microorganisms may not be sufficient when considering microbial quality, especially in reuse for irrigation, and may require a post-treatment of wetland effluents in order to achieve an adequate reduction of the pathogen microorganisms through for example chlorination or UV disinfection.

2.5. HSSF-CWs Performance Modelling

Pollutants removal in HSSF-CWs involves a number of different processes which are of high complexity influence each other and can take place at the same time and at different sites within the bed (Kadlec & Wallace, 2009; Garcia *et al*., 2010; Vymazal, 2010). This complexity may explain the difficulty in understanding all interactions that may occur within the system between all its components and has led to the fact that they are seen for a long time as a “black box” (Rousseau *et al.,* 2004; Faulwetter *et al*., 2009). However, over time it was tried to develop numerical models that could simulate the removal of pollutants from CWs and to be used as predictive process design tools (Langergraber *et al*., 2009).

Different types of models with diverse complexity have been developed to simulate the processes of pollutant removal in wetland systems and they include from the relatively simple design models like “rules of thumb” and regression equations along with first-order kinetic models to more complex and dynamic models (García *et al.,* 2010; Liolios *et al.*, 2014). So, these models may be numerical, statistical and during the past few years, many models for processes occurring in a CW have been described based on complex and dynamic software.

Rules of thumb are based on observations from CWs and have been used for estimated the area of a given wetland system in order to obtain a specific quality of the final effluent for some wastewater quality parameters. However, since this rules of thumbs are based on observations from wetland systems that are located at a wide range of climate and which use different types of vegetation and treating different kinds of wastewater, they have low precision in predictive capacity and should be used only as a validation method to other sizing methods such as the first-order kinetic model k-C* (Rousseau *et al.*, 2004). These methods are usually referred to the mass of organic loading that must be applied per unit area of wetland system, or are sized taking into account a certain TRH or based on the volume of wastewater to be applied per unit size of systems (i.e. HLR).

Generally, the HSSF-CWs are sized according to the International design criteria and experience (Kadlec 2002; Vymazal, 2010; Wallace & Knight, 2006), which take into consideration that most of the biological and chemical treatment can occur within a 5 to 14 days of residence time within the system, while HLR considered suitable for the treatment of domestic wastewater within those systems range between 2 to 20 cm per day. Regarding to the organic loading rate applied to these systems, usually are suggested values ranging between 2 to 12 gBOD.m^{-2}.day and 5 to 20 gCOD.m^{-2}.day in order to guarantee that the wetland effluent complies with the quality standards required by legislation (e.g. ≤ 25 mgBOD.L^{-1} in the case of Portugal). According to the same criteria, the solids loading rate applied to HSSF-CWs should range between 5 to 12 gTSS.m^{-2}.day in order to minimize bed clogging. One of the most widely used rules of thumb is based on the specific surface area required per equivalent inhabitant, and is usually suggested a value of 3 to 6 m^2/p.e.

The first-order k-C* degradation model was developed by Kadlec & Knight (1996) and are based on area-based removal rate constant (k), flow rates and pollutants concentrations of influent. These models consist of first-order equations which assuming a plug-flow hydrodynamics and constant conditions (*e.g.* flow and concentrations) and which predict that many pollutants decline exponentially to a background concentration (C*) upon passage through a wetland (Rousseau *et al.,* 2004; Langergraber *et al.,* 2009; Kumar & Zhao, 2011).

However, first-order kinetic models assumes that all fluid particles have a uniform HRT, nevertheless some studies that applied tracers have shown that occurs dispersive processes due preferential pathways and pore friction and they also point out that first-plug flow models not take into account the many complex reactions that occur within the systems (Garcia *et al.,* 2004). Kadlec (2002) itself also considered this model inadequate due primarily to the variability of several factors such as input flows and loads, precipitation and evapotranspiration which can cause temporary deviations from the steady state conditions assumed by this simple model. In fact, any of those factors can change the HRT and dilute or concentrate the pollutants in wastewater, which may have a decisive effect on system performance.

As plug-flow hydraulic model fail to accommodate dispersive processes within HSSF-CWs, for modelling hydraulic performance of these systems, the Tanks-in-Series model has been steadily gaining recognition (Kadlec & Wallace, 2009). This model approach considered that a HSSF system may be approximated to a series of hypothetical completely mix and continuous stirred tanks, determining the degree of dispersion by tracer studies (Wallace & Knight, 2006).

The HSSF-CWs treatment performance and effluent pollutant concentration also has been assessed and predicted by statistical models including those derived from using linear and multiple regression analyses, which use wastewater quality parameters that are easily determined *in situ* and its accuracy in predicting the performance of the system has been demonstrated (Vymazal, 2010). These models provide estimates regarding change in parameter concentration and/or loading, despite treats wetland as a black box since they are generally based on input and output concentrations or loadings (Kadlec & Wallace, 2009).

Rousseau *et al.* (2004) reviewed current CWs design approaches and conclude that, despite the obvious deficiencies, the first-order plug flow model remains to be the most acceptable model in design of HSSF-CWs and to be used for fitting BOD_5, COD and TSS removal data, and that is also consistent with said by Kadlec & Wallace (2009) and Trang *et al*. (2010). Rousseau *et al.* *(*2004) also state that rule of thumb methods were much widespread and that often overestimate the size of the beds, and they concluded that the regression equations often have limited value given the wide variability in the equations that have appeared in the literature, which is normally a consequence of different specific conditions (*e.g*. climate, bed material, physical dimensions of the wetland bed and HLR) under which they were obtained.

Recently, numerical models for CWs became promising tools for a better description and an improved understanding of CW treatment processes and performance (Langergraber *et al*., 2009; Samsó, 2014). Some numerical models with different complexities are available based on a set of mathematical expression (algebraic or differential equations) each describing the biochemical reactions that are responsible for the degradation and transformation processes that take place within the CWs. (Llorens *et al.* 2011; Samsó, 2014). Compared to the empirical models, these models are more versatile and accurate predictive tools over a much higher range of operating conditions and allow the testing of sensitivities of the pollutant removal processes in different operational conditions when the evaluated scenarios cannot be easily tested physically (Bezbaruah & Zhang, 2004; Rousseau *et al.,* 2004; Mburu *et al*., 2013; Liolios *et al.,* 2014).

The most commonly used models for describe HSSF-CWs are numerical models and that are explained in details by Langergraber *et al.* (2010) and they involved the use mathematical expressions (algebraic or differential equations) that have been proved very valuable for understanding, quantifying and even predicting the complex processes that take place within constructed wetlands. Details for several models applied to the removal of a specific pollutant or a set of pollutants or that is focusing on the simulation of the hydraulic or hydrodynamic of the system can be found in the literature (*e.g.* Mayo & Bigambo, 2005; Akratos *et al*., 2008; Galvão *et al*. 2010; Knowles *et al*., 2011; Kumar & Zhao, 2011; Samsó, 2014).

Rousseau *et al.* (2004) considered that the major limitations of these models is related to its own complexity and the need for a wide range of data of various parameters, which often do not exist for most of the sites where we want to install treatment wetlands systems. Thus, according to the same authors for lack of site specific parameter data, there is often a tendency to use most typical parameter data to satisfy the requirements of the model, which does not seem to contribute to increased precision at the level of tools to evaluating and improving the existing design criteria than that would achieve using a first-order model.

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3. Performance evaluation of three horizontal subsurface flow constructed wetlands in the Interior Central Region of Portugal during the summer period[1]

Presented in Joint Meeting of Society of Wetlands Scientists (SWS), WETPOL and Wetland Biogeochemistry Symposium, 3-8 July 2011, Prague, Czech Republic (oral presentation, published in CD-Rom).

Abstract

Constructed wetlands (CWs) have been showing to be effective, low cost, economical and sustainable alternative solutions for wastewater treatment in comparison with conventional technologies, mainly for small rural communities. This study was carried on in three gravel based horizontal subsurface flow CW located at Aranhas, Capinha and Janeiro Cima (rural areas of the Interior Central Region of Portugal), between May and August 2008 (summer period). The objective was to evaluate the performance of the CW in the removal of pollutants and pathogens, approximately 4 years after the start-up. The summer period was chosen since is the season that may present more problems for CW performance due to high evaporation rates and high variation of incoming loads. The hydraulic loading rates (HLR) were 7.6, 3.0 and 5.5 $cm.d^{-1}$ for Aranhas, Capinha and Janeiro de Cima, respectively. Generally, the results show that both the organic and the solids loads had a high variation during the summer period, whilst the influent loads of nitrogen and phosphorous were low. The mean removal efficiency (RE) for COD was 59% (Aranhas), 75% (Capinha) and 76% (Janeiro de Cima), which are lower than the values found in similar systems in Mediterranean countries. However, the beds showed a good filtration capability (RE for TSS of 79%, 94% and 84% for Aranhas, Capinha and Janeiro de Cima, respectively). The mean removal of nitrogen was very low in all systems (< 27% for TN, < 30.8 for org-N, < 7% for NH4+-N and < 18% for NOX-N), although the plants were well developed and the hydraulic retention time 6.6 d (Aranhas), 17 d (Capinha), 9 d (Janeiro de Cima) was quite high to promote nitrification. However, the RE for TP was high 44.3% (Aranhas), 41.2% (Capinha), 65% (Janeiro de Cima). TSS and COD RE was found to significant linearly proportional to its influent mass loading rate ($p<0.05$). Linear regression also showed statistically significant ($p<0.05$) increase in outlet concentration of TSS and TN with increasing its mass loading rates. Similar results were seen between NH4+-N and TP effluent concentration and its inlet loads, despite this relationship to be only significant

[1] Mesquita, M.C; Latado, M.; Carreiro, F; Albuquerque, A; Amaral, L.; Nogueira, R., (2011). “Performance evaluation of three horizontal subsurface flow constructed wetlands in the Interior region of Portugal during the summer period” (published in CD-Rom). Oral presentation in Joint Meeting of Society of Wetlands Scientists (SWS), WETPOL and Wetland Biogeochemistry Symposium, 3-8 July 2011, Prague, Czech Republic.

($p<0.05$) for Aranhas and Janeiro Cima systems, respectively. Generally, the final effluent from the Three CWs complies with the Directive 271/91/EEC guidelines.

Keywords: Constructed wetland; Horizontal subsurface flow; Loading rate; Removal efficiency; summer period.

3.1. Introduction

In Europe, the last two decades witnessed growing water stress, both in terms of water scarcity and quality deterioration, which prompted many municipalities not only for a more efficient use of the water resources, but especially for a more efficient wastewater treatment. Municipal wastewater is one of the major concerns of the environmental problems (Song *et al*., 2006) and therefore, wastewater treatment is essential to minimize the effect of the contaminants to nature. Constructed wetlands (CWs) are regarded today as key elements in treat or polishing municipal wastewater and others different types of wastewaters. They are biological systems which applies the interaction between media, plants and microorganisms during the degradation of pollutants present in the wastewater and that have been proven to be effective, low cost, economical and sustainable alternative for conventional wastewater treatment technology (Vymazal & Kröpfelová, 2008), mainly for the treatment of domestic sewage from small villages and rural communities, frequent situation at the Interior Central Region of Portugal.

The advantages of using these systems in this region of the country, and in other rural areas of Portugal, are due to favourable climatic conditions such as temperature, and relative availability of land in those regions that determine relatively low costs concerning its acquisition. Moreover, this technology could also be a viable solution for promoting reuse of treated wastewater in agriculture since that is in these rural areas that much of agricultural activity is concentrated.

The growing public awareness and acceptance in Portugal of CWs have increased significantly the number of these facilities with the objective of treating wastewater from small agglomerates. Although there are no recent data, a national inventory data until July 2006 indicates a total of 304 operating systems, which included system of different sizes, from small uni-housing systems with an average flow of less than $1m^3\ d^{-1}$ (176), to municipal systems with capacities ranging from 30 to 12,000 population equivalents (p.e.) (128) (Ribeiro, 2007). However, it is believed that the number of existing CWs in Portugal is likely to be higher than those.

Most systems are built in Portugal based on macrophytes emerging beds of horizontal subsurface flow (HSSF), with the aim of obtaining a secondary treatment stage. Regarding the type of treated wastewater, the majority have been installed to treat domestic or municipal wastewater (Albuquerque *et al.*, 2008; Oliveira, 2008). Although the most used macrophytes is the common reed (*Pragmites australis*), in some cases it is possible to find *Typha* spp..

Several studies on the treatment efficiency and capacity of full-scale HSSF-CW have been published (Vymazal, 2002; Chiemchaisri *et al*., 2006; Song *et al*., 2006; Brix *et al*., 2007; Vera *et al*., 2011). Generally, these studies show that removal efficiencies of total suspended solids (TSS), biochemical oxygen demand (BOD_5), Chemical oxygen demand (COD) and pathogens are generally high, whereas removal percentages of nutrients (nitrogen (N) and phosphorus (P)) are often lower and more variable. Data from the literature have shown removal efficiency (RE) of BOD that is above 60%, reaching values above 85% in many studies (Solano *et al.,* 2004;

Headly *et al.,* 2005; El-Hamouri *et al.,* 2007). Regarding COD, it appears that most of the values reported in the literature vary between 50% and 90% (Vymazal, 2001; Garcia *et al.,* 2005; Akratos & Tsihrintzis, 2007). With respect to the TSS, RE is usually high in HSSF-CWs and several studies shows that the values are generally higher than 80% (Vymazal*,* 2001; Karathanasis *et al.,* 2003; Solano *et al.,* 2004).

Regarding the RE of nitrogen, many studies report rates of removal of total nitrogen (TN) very low, often below 55%, even when the removal efficiencies of ammonium-nitrogen (NH_4^+-N) are in the order of 70% (Vymazal, 2007; Chung *et al.,* 2008; Galvão, 2009).

In Catalonia (Spain), the performance evaluation of eight years experience of CW systems treat wastewater from small communities, indicating that BOD_5 concentration was below 35 mg.L^{-1} and removal efficiencies were between 65% and 88%, while for the nutrients, the RE for TN and total phosphorus (TP) were in the range of 48%-66% and 39-58%, respectively (Vera *et al*., 2011). For the Czech Republic and for HSSF systems, Vymazal (2002) reported a mean effluent TN concentration of 27 mg.L^{-1} and removal efficiencies of 42% and Brix *et al*. (2007) referred for Danish HSSF wetland systems, removal efficiencies for TN and TP ranging between 30 and 50%. Chiemchaisri *et al.* (2006), in a HSSF-CW treating young leachate (Bangkok, Thailand) achieved a high organic removal at 94% and 98% in terms of COD and BOD_5, respectively, and moderate TSS removal (71-88%). The last authors, in term of nitrogen removal, obtained average TN removals of 43%. In ten monitored Italian full-scale HSSF-CW systems applied as secondary treatment plants for municipal and domestic wastewater and a hydraulic retention time (HRT) between 3-4 days, Masi *et al.* (2000) obtained an average overall treatment RE of 83.7%, 86.1%, 64.0% and 94.5% for COD, TSS, NH_4^+-N and for nitrate-nitrogen (NO_3^--N), respectively.

The evaluation of CW facilities performance, under Portugal conditions, are still recent and has not be extensively reviewed, despite the data already obtained and discussed, confirming the general tendency for the potential treatment efficiency of these systems with higher reduction levels of BOD_5, TSS and pathogens and less efficiency in regard to the removal of nitrogen and phosphorus (Dias *et al.,* 2006; Oliveira, 2007; Galvão, 2009). However, it is important to carry out the monitoring and collecting data of sufficient detail from as many systems as possible, in order to analyze empirical data collected and to obtain a consistent and comprehensive database concerning to Portuguese installed systems.

Therefore, the objective of this study was to evaluate the performance of the three CWs located at the Interior Central Region of Portugal in removing of pollutants, approximately 4 years after the start-up. The summer period was chosen since is the season that may present more problems for CW performance due to high evaporation rates and high variation of incoming loads related to the floating population that significantly increases in the zone studied during the summer holidays. The paper also discusses the relationships between loading rates and removal efficiency and effluent concentrations in order to better clarify those results.

3.2. Materials and methods

3.2.1 Study site and constructed wetlands description

The CWs systems under investigations are situated in Beira Interior Region of Portugal (rural areas of the Interior Central Region of Portugal) and are located at villages Aranhas, Capinha and Janeiro de Cima (Figure 3.1). The Aranhas system is situated at a longitude of 7º22’49.65‘’ W and a latitude of 40º11’33.99’’ N, near to Penamacor town. Capinha and Janeiro Cima are of coordinate’s 7º22’49.65’’ W and 7º48’5.04’’ W of longitude and 40º11’33.99’’ N and 40º3’49.01’’ N of latitude, respectively. The two systems are near the town of Fundão (Figure 3.1).

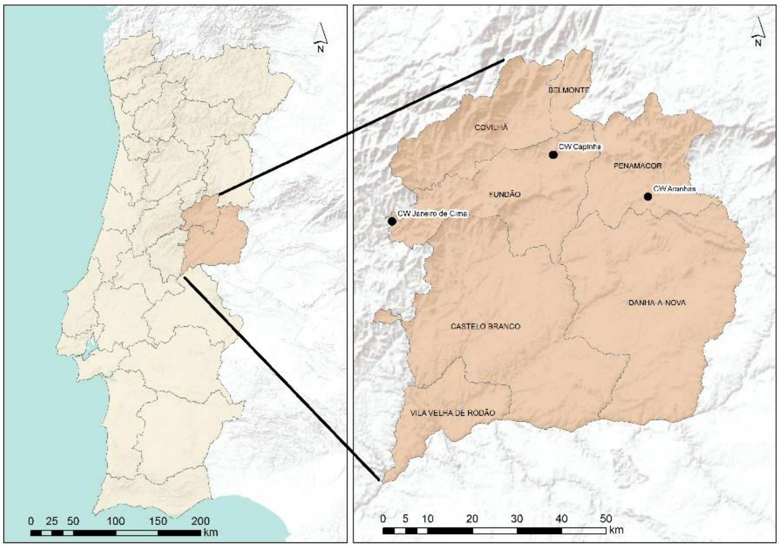


Figure 3.1 Geographical locations of the study systems

The area is characterized by a climate temperate Mediterranean, with marked continental effect, with high temperature ranges and almost all of the rainfall concentrated in autumn/winter (Costa, 2006). Climatic characteristics of the region also highlight the fact that summer is normally very dry and the average annual temperature varied between 12.5 ºC and 15 ºC for Aranhas system and 10 ºC and 12.5 ºC for Capinha and Janeiro de Cima (Costa, 2006). According the same author, the average annual precipitation ranged from 700 to 800 mm for Aranhas and 800 -1000 mm for Capinha and Janeiro Cima.

This study was carried out between May and August 2008 (summer period), during which the air temperature ranged from 16.8 ºC to 23.4 ºC and the precipitation varied between 0.0 mm and 57.4 mm. The studied systems mainly receive domestic wastewater, as well as effluents from septic tanks that serve some rural villages nearby and private homes and small agricultural industries.

The Wastewater Treatment Plant (WWTP) of Aranhas presently serves a population of 437 inhabitants and the preliminary treatment includes bar racks, screening and flow measurement followed by primary treatment in a septic tank. The secondary treatment includes a single bed (642 m^2) with a specific surface area (SSA) of ~1.5 m^2/p.e. concerning the systems of Capinha and Janeiro Cima, they currently serve a population of 521 and 349 inhabitants, respectively. In both systems, preliminary treatment is the same to that observed in WWTP of Aranhas. However, these systems have an Imhoff tank as a primary treatment. The treatment system in Capinha and in Janeiro Cima treat domestic wastewater on a surface area of 1 550 m^2 (SSA = ~3 m^2/p.e.) and 1 060 m^2 (SSA = ~3 m^2/p.e.), respectively. All beds were colonized with Phragmites australis and the water level was 0.5 m. The bottom of all beds was covered with impermeable material (HDPE) and they were filled with gravel (bottom layer, 20 cm), followed by a layer of sand (30 cm) and, finally, a layer of topsoil (first 10 cm).

The influent flow-rate was measured daily and the mean flow-rate was 49, 45 and 58 $m^3.d^{-1}$ for Aranhas, Capinha and Janeiro Cima, respectively, which corresponds to hydraulic loading rate (HLR) of 7.6, 3.0 and 5.5 cm. d^{-1}, respectively.

3.2.2. Sampling procedure and analytical methods

Biweekly water samples were collected manually at the raw inflow (after screen bars) and at the inflow (after primary treatment) and outflow of CW system over a period of three months. The samples were normally sampled between 8 a.m. and 10 a.m. in the sampling day. Samples were transported at 4 ºC to the laboratory and the following parameters were analyzed within 24 h of sample collection: pH, COD, TN, organic nitrogen (Org-N), NH_4^+-N, oxidized nitrogen (NO_x-N) that is summation of nitrite nitrogen (NO_2^--N) and nitrate nitrogen (NO_3^--N), TP and TSS. Water samples were analyzed in accordance with the Standard Methods for Examination of Water and Wastewater (APHA-AWWA-WEF, 2005). The pH were measured by potentiometric method, COD by using potassium dichromate method in open reflux conditions, TSS were determined through gravimetric method-filtering with GF/A filter paper and residue dried at 103-105 ºC. Total nitrogen was determined by titrimetric method, after digestion and distillation of the sample (Macro-Kjeldahl Method), while the determination of NH_4^+-N used the titrimetric method, after distillation the sample. NO_x-N was determined by Devarda's alloy reduction method. Organic nitrogen was estimated by subtracting the concentrations of ammonium and oxidized form total nitrogen. TP were measured by persulfate digestion followed by ascorbic acid method.

It was not possible to continuously monitor the variable flow rate in the outlet, therefore for all parameters the removal efficiency was determined from concentration and not from loadings and was calculated as difference of concentration in influent ($C_{infl.}$) and effluent ($C_{efflu.}$) to the system divided by concentration in influent and multiplied by 100 per cent.

3.2.3. Statistical analysis

All statistical analyses of the data were completed by using Microsoft Excel v.2007 Analysis ToolPak. Data were analyzed for Analysis of Variance (ANOVA) for determining the differences between sample characteristics before and after treatment collected from raw influent, inlet and outlet of the treatment plants and the significance level of 0.05 was used in the ANOVA (Analysis of variance) and in the linear regression tests.

3.3. Results and discussion

A summary of the average concentration and standard deviation (SD) values of different physical-chemical parameters analyzed either in raw influent or in inlet and outlet of the three CWs systems, during the monitoring period, are presented in Table 3.1.

Table 3.1 - Characteristics (mean ± standard deviation) of raw influent, inlet and outlet for three constructed wetlands analyzed**.**

Parameters	Aranhas			Capinha			Janeiro de Cima		
	Raw Inf.	Inl. CW	Out. CW	Raw Inf.	Inl. CW	Out. CW	Raw Inf.	Inl. CW	Out. CW
pH	6.9±0.3	6.8±0.3	6.8±0.2	6.9±0.5	7.0±0.2	6.9±0.2	7.1±0.3	6.9±0.2	6.9±0.4
TSS (mg/L)	339±122	185±55	43±9	1077±269	525±305	33±18	412±203	227±155	39±11
COD (mg/L)	497±293	213±106	88±43	983±805	357±184	90±41	755±382	278±167	67±23
TN (mg/L)	10.1±2.5	8.2±2.8	7.8±2.0	16.3±6.1	12.6±2.9	11.2±4.0	13.5±2.1	10.5±3.5	10.6±2.9
Org –N (mg/L)	1.4±0.4	1.3±0.4	0.9±0.2	1.7±0.2	1.6±0.2	1.2±0.6	1.5±0.5	1.3±0.5	1.2±0.6
NH_4-N (mg/L)	7.8±2.7	6.2±3.4	6.2±2.0	13.9±6.1	10.2±3.0	9.5±5.6	11.4±2.0	8.6±3.4	9.0±2.7
NO_X-N (mg/L)	0.9±0.3	0.7±0.5	0.7±0.3	0.7±0.3	0.8±0.7	0.5±0.4	0.6±0.3	0.6±0.8	0.4±0.3
TP (mg/L)	5.1±4.1	4.7±2.7	1.9±1.1	7.8±2.6	6.3±2.8	3.1±1.9	5.2±1.8	6.5±2.3	2.1±0.7

Note: raw influent (Raw Inf.), inlet (Inl.) and outlet (Out.)

3.3.1. pH

The mean values both in raw influent as in inflow at the all CWs are close to 7units as shown in Table 3.1, indicate neutral conditions in the treatment systems during the study period. The inflow CWs ranges observed for pH was 6.4-7.2 (Aranhas), 6.8-7.1 (Capinha) and 6.7-7.1 (Janeiro Cima). The observed ranges of inlet pH are within the recommended range (4<pH<9.5) for the existence of the most treatment bacteria, nitrifies and denitrifies as well as for the growth and development of macrophytes (Kadlec & Knight, 1996).

Although no significant differences ($p<0.05$) have been observed between outlet pH and raw and inlet effluent pH, generally, the results showed that there was a slightly tendency to a pH reduction during the passage through the beds. This decrease in pH could have resulted from potential nitrification process. However, probably the buffer capacity of the wastewater did not always allowed detecting the decrease in pH.

For the systems study, the average effluent pH during the whole monitoring period ranged 6.6 to 7.2. The values obtained fall within the range permitted by the Portugal quality standard for discharging in freshwater (6.0-9.0) and satisfy the goal recommended for the effluent reuse in irrigation (6.5-8.4).

3.3.2. Total Suspended Solids (TSS) removal

Raw influent presented a high suspended solid concentration as indicated by average values (Table 3.1), especially in case of Capinha system and the results also show that the concentration of TSS were very variable as shown the high SD of the mean values. The mean TSS concentration of raw influent on Aranhas and Janeiro Cima fall under the class of strong sewage, while for Capinha the mean value obtained is high compared with the typical value of ~400 $mg.L^{-1}$ reported for strong domestic wastewater (Metcalf & Eddy, 2003). The high values obtained in Capinha WWTP can be explained by the discharge into the local sewer of wastewater from small agro-industries, especially small cheese factories and by discharge of the content from the cleaning of septic tanks that serve small rural villages nearby. In spite of high concentration of TSS in the raw inflowing sewage, the primary treatment worked properly and provided about 44.2±10.5%, 53.4±20.3% and 44.1±31.3% of TSS removal in Aranhas, Capinha and Janeiro Cima, respectively.

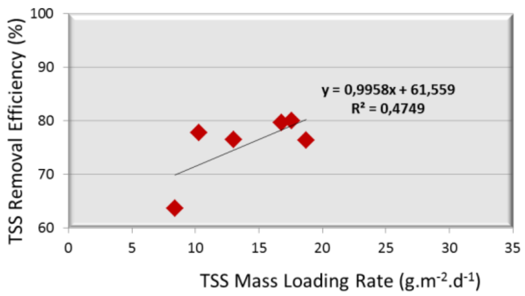
TSS are removed by physical processes such as sedimentation and filtration, followed by aerobic and anaerobic degradation (Kadlec & Knight, 1996) and its removal is generally high in systems with horizontal subsurface flow (Vymazal, 2011) and the CWs studies are no exception and have been shown a good filtration capability. In fact, In spite the SSA values are lower than reported by (Vymazal, 2005) that established that is necessary for eliminate TSS an unit area of 5 m^2/p.e. and by (Rousseau *et al*., 2004) that indicate for HSSF systems treating municipal sewage that a unit area between 4-8 m^2/p.e. is required to obtain an effluent that respect the TSS limits of Urban Wastewater Treatment Directive (91/271/EEC), the mean removal efficiencies for TSS obtained on systems studies are consistent with the values reported in literature (Vymazal, 2002; Puigagut *et al*., 2007; Albuquerque *et al*., 2009; Vera *et al*., 2011).

The average removal efficiencies indicate that the required conditions for those processes were reached in all three systems, conducting mean overall RE of TSS of about 77% (Aranhas), 94% (Capinha) and 84% (Janeiro Cima). However, when considering systems individually, a relation between the unit area and the removal percentages could be establishing. The Capinha and Janeiro Cima plants with larger SSA (~3 m^2/p.e.) present a RE higher than Aranhas system with a smaller SSA (~1.5 m^2/p.e.).

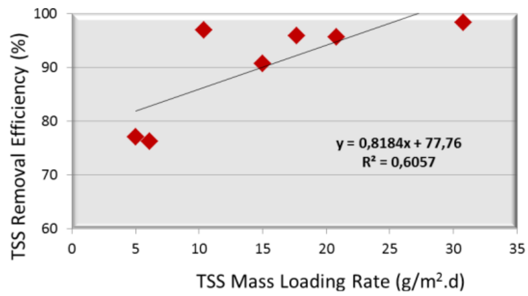
Despite the big fluctuations in inlet concentration of TSS over monitoring period, the removal efficiency did not show this tendency and was similar along the time for all systems and these results are consistent with those of other authors (Masi & Martinuzzi, 2007; Albuquerque *et al*., 2008) and showed that the HSSF-CWs systems tend to present good resilience against significant changes in the solids loading rate applied.

TSS loading rates ranged from 8.4 to 18.7 gTSS m^{-2} d^{-1} (Aranhas), 5.0 to 30.8 gTSS m^{-2} d^{-1} (Capinha) and 6.0 to 28.1 gTSS m^{-2} d^{-1} (Janeiro Cima). The mean values obtained for TSS loading rate were 14.1±4.2 gTSS m^{-2} d^{-1} (Aranhas), 15.2±9.0 gTSS m^{-2} d^{-1} (Capinha) and 12.4±8.5 gTSS m^{-2} d^{-1} (Janeiro Cima) and we verified for all systems that were higher than the inflow loads of TSS between 5 and 12 gTSS m^{-2} d^{-1}, recommended by international design criteria (USEPA, 2000; Vymazal, 2003) in order to avoid problems in porosity and subsequent changes in hydrodynamic behavior which could affect the removal efficiency of TSS.

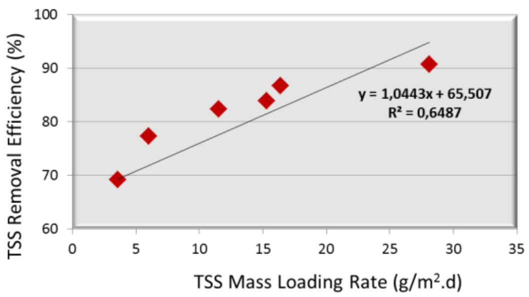
The influence of the mass loading of TSS on its RE in each system were evaluated graphically as shown in Figure 3.2. The mass loading rate was computed as the product of inlet pollutant concentration and the HLR. There were a significant ($p<0.05$) effect of TSS loading rates on its removal efficiency for all systems and that was found to be linearly proportional to its mass loading rate with coefficient of determination (R^2) of 0.4749 (Aranhas), 0.6057 (Capinha) and 0.6487 (Janeiro Cima) and apparently these two variables for all systems had a positive significant correlation (p= 0.000; r= 0.689 for Aranhas; p= 0.000; r= 0,778 for Capinha and p=0.000; r= 0.805 for Janeiro Cima) The close fit of the points to the regression line also indicate a remarkably constant relationship between the two variables. Thus, under summer conditions the results seems to indicate that one can add more TSS within a certain range to a HSSF-CW without diminishing removal efficiency at least up to a certain point where clogging problems may start to arise (Tanner *et al*., 1998)



a)



b)



c)

Figure 3.2 Relationship between TSS removal efficiency and TSS surface mass loading rate: (a) Aranhas System; (b) Capinha System; (c) Janeiro Cima System.

It is also observed that there is a reasonable linear relationship between TSS concentration in effluent of wetlands and its influent mass loading rates, with a linear regression coefficient (R^2) of 0.6913 (Aranhas) and 0.4928 (Janeiro Cima), with highest effluent TSS concentration associated with high influent loading rate, indicating that TSS concentration in the effluent of these CWs will be statistically significant linearly proportional to their influent mass loading rate. For these systems those two variables had a significant correlation with p value less than 0.05 and with Pearson Coefficient of 0.831 (Aranhas) and 0.702 (Janeiro Cima). However, for Capinha system we observed that there was a negative linear relationship between these two variables, although of this relationship was not significant ($p>0.05$).

The average effluent values were 43.0±9.0 mg.L^{-1} (Aranhas), 32.7±18 mg.L^{-1} (Capinha) and 37.0±11 mg.L^{-1} (Janeiro de Cima), which are high when compared to the average TSS value of 10.2 mg.L^{-1} reported by Vymazal (2002) or by Cooper (2009) which referred a TSS concentration below 30 mg.L^{-1} after year 4, for a full-scale HSSF system used as secondary treatment in England. Brix *et al.* (2007) also achieved, for Danish HSSF systems effluent concentrations of TSS, values lower than 20 mg.L^{-1}. The difference with the data reported by these authors could be due to the higher influent TSS concentration observed on three systems studies that may be resulting in higher outflow concentration. Other possible explication for this difference is the solid accumulation in the wetland input zones and a possible relation between this accumulation and the discharge values (Caselles-Osorio *et al.,* 2007). However, our results are consistent with the values (52 mg.L^{-1}) reported by Arendacz *et al.* (2008) and by Marecos do Monte & Albuquerque (2010) (34.0 ±10.0 mg.L^{-1}).

The TSS concentration of treated effluent was found to be significantly ($p<0.05$) different from raw and inlet concentrations and always met the national discharge standards established for systems serving population equivalent< 2000 inhabitants, corresponding to a maximum concentration of 60 mg.L^{-1} (Decree-Law No. 236/98 of 1 August). However, in spite of good removal rates observed, the outlet TSS concentration does not have always satisfied the recommended limit of Urban Wastewater Directive (271/91/EEC) (35 mg.L^{-1}). In fact, during the study period, TSS were release at levels above recommended limit of 35 mg.L^{-1} in 83% of the samples in Aranhas system, in 43% and 71% of the samples for Capinha and Janeiro Cima, respectively. However, all systems met the minimum percentage of TSS removal established at that directive (> 70%).

If effluent is intented to be reused for irrigation, achieving high TSS removals is important to avoid the clogging of soil pores and irrigation dives. In the present study, the concentrations of TSS in the effluent for all systems were below the required quality standard of water for irrigation (60 mg.L^{-1}), according Portuguese legislation (Decree-Law No. 236/98 of 1 August).

3.3.3. Chemical oxygen demand (COD) removal

COD is an important parameter to assess the quality of effluents and is also used for monitoring and control of discharges and for assessing treatment plant performance. Generally, the results show that the organic loads had a high variation during the summer period, especially at Capinha system, as shown by the high values obtained for the standard deviation (Table 3.1). This fluctuation in raw influent concentrations values is probably mainly due to the discharge into sewer network of wastewater from small agro-industrial activities which are unable to treat their own effluents for economical reasons and, as already noted, by occasional discharge into WWTP the content from the cleaning of septic tanks that serve small rural villages nearby, whose composition is highly variable and difficult to draw the typical values for concentrations of pollutants.

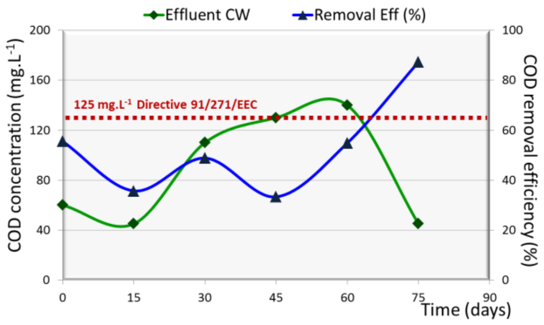
Primary treatment removes on average 54% of COD in Aranhas WWTP and 56% and 59% of COD in Capinha and Janeiro Cima WWTPs, respectively. Although the primary treatment could have acted as a buffer to attenuate the variation of incoming loads to the beds, it was detected an oscillation at COD concentration in the bed inlet for all CWs (Table 3.1). Consequently, the results show that the OLR applied to the beds also had a high variation during the study period. In fact, for Aranhas system the OLR applied varied between 5.3- 27.1 gCOD $m^{-2}.d^{-1}$, while for Capinha and Janeiro Cima it was observed a range of values between 3.8-17.4 and 7.7- 35.0 gCOD $m^{-2}.d^{-1}$, respectively.

As shown at Table 3.1, organic concentrations expressed as COD can be reduced by macrophyte systems. This removal efficiency reached average levels of 59% (Aranhas), 75% (Capinha) and 76% (Janeiro de Cima) (Figure 3.3). The differences on performance between the systems studies can be attributed to the different average organic input load, higher in Aranhas (16.3±8.1 gCOD $m^{-2}d^{-1}$) which is close to the maximum sizing criteria suggested by the German guideline (16 gCOD $m^{-2}d^{-1}$) (Albuquerque *et al*., 2009). This can be attributed to its lower specific surface area (~1.5 m^2/p.e), compared to the others systems that presented a SSA twice (~3 m^2/p.e) higher than the Aranhas. The average removal efficiency in terms of COD agrees with the results by Albuquerque *et al.* (2009) who employed OLR comparable (9.4-22.3 gCOD $m^{-2}.d^{-1}$). Nevertheless, Masi & Martinuzzi (2007) attained 94% of COD removal with OLR of 2.2 to 34.1 gCOD $m^{-2}.d^{-1}$.

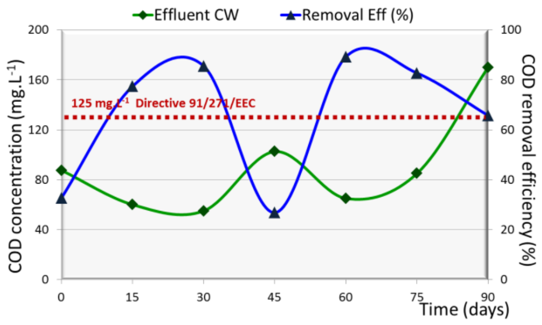
Removal efficiencies observed seems to be lower than the ones found in similar systems in Mediterranean countries and other region of Europe, which could be associated to the discharge of influents of agro-industries and others types of influents that increased the particulate hard-to-biodegraded organics (more than 50% of the total COD in the influent as observed in a previous study by Albuquerque *et al.* (2009) or may be resulting from lower availability of the effective area for organic removal due to the use of inappropriate sizing criteria.

The COD was decreased significantly ($p<0.05$) in treated effluent compared to the levels observed at the entrance of beds and despite the significant variation observed at the inflow concentration of the CWs and, consequently, on the OLR, the results show that the limit concentration imposed by the Directive 91/271/EEC was exceeded just in two samples in Aranhas and in one sample in Capinha (Figure 3.3). However, according to the legislation in Portugal, the emission limit value established for agglomerates with an equivalent population < 2000 inhabitants it is of 150 $mg.L^{-1}$ and the results show that this value has been exceeded only once in the WWTP Capinha. The mean COD concentration decreased from 213±106 to 88±43 $mg.L^{-1}$

(Aranhas), 357±184 to 90±41 mg.L^{-1} (Capinha) and 278±167 to 67± 23 mg.L^{-1} (Janeiro Cima). In terms of reused effluent for irrigation, there is not any COD limit established in Portugal.



a)



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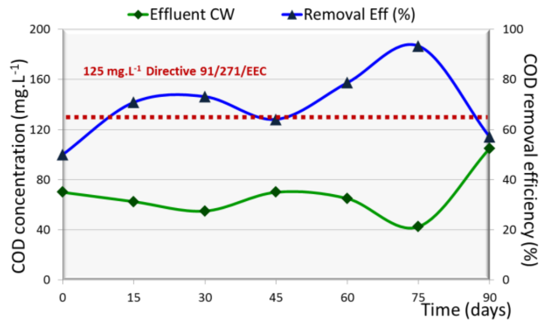


c)

Figure 3.3 Temporal variation of effluent COD concentration and COD removal efficiency along the monitoring period: (a) Aranhas System; (b) Capinha System; (c) Janeiro Cima System.

A regression analysis between COD effluent concentrations and inflow COD mass loading rates do not provide a good relationship. Although the result showed higher effluent COD concentration with higher COD mass loading rate, the coefficient of determination (R^2) were very weakly for all systems (Aranhas:R^2=0.0818; Capinha: R^2=0.0086; Janeiro Cima: R^2=0.2459). Thus, apparently there was no significant effect of COD loading mass rates on its effluent concentration. However, the influence of the mass loading of COD on its removal efficiency in each system were evaluated and good positive linear proportional relationships was seen between this two variables (Figure 3.4), with a regression coefficient of 0.5503 (Aranhas), 0.6679 (Capinha) and 0.7041 (Janeiro Cima), showed highest removal efficiency associated with high influent COD loading rate, despite this relationship was significant ($p<0.05$) just for Capinha and Janeiro Cima systems.

A significant correlation was also seen between these two variables with *p-values* less than 0.05 for all systems (p=0.000; r=0.742 for Aranhas, p=0.000; r=0.817 for Capinha and p=0.000; r=0.839 for Janeiro Cima). The results obtained seems to indicate that the HSSF-CWs systems tend to present good resilience against significant changes in the OLR and one can add more

COD under summer period without declining removal efficiency. However, it is expected that above a certain concentration in the affluent, problems of clogging and overflow beds might arise, which probably will result in a decrease in the removal rate.

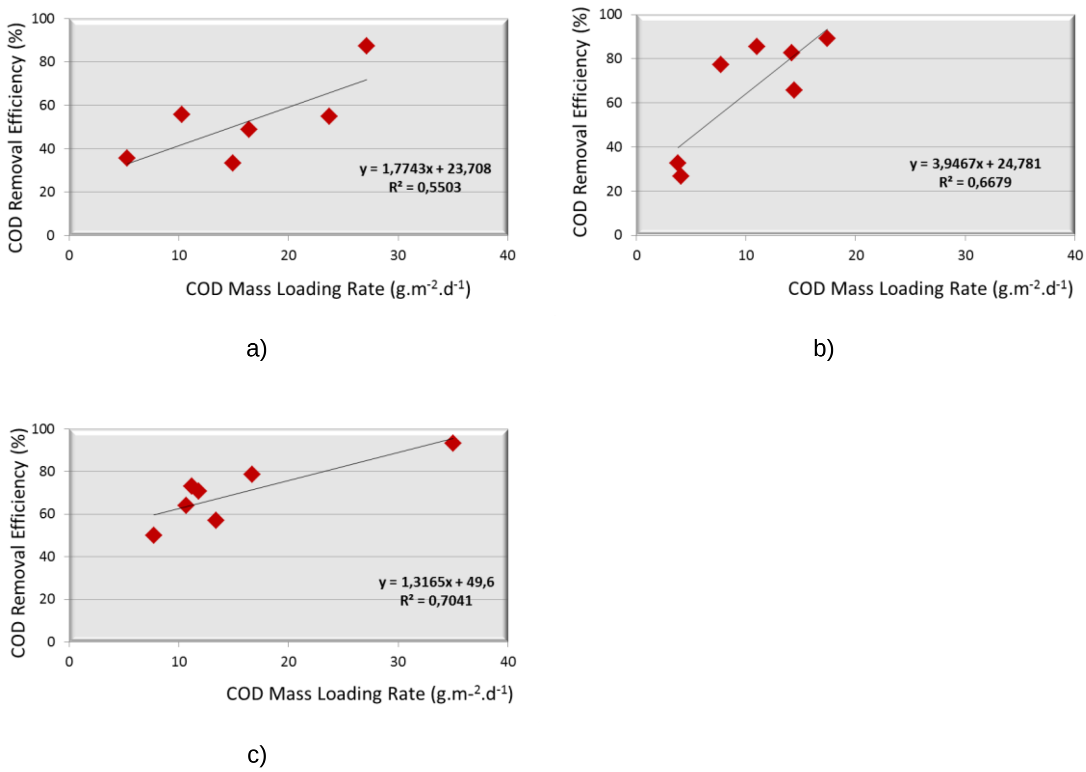


Figure 3.4 Relationship between COD removal efficiency and COD surface mass loading rate: (a) Aranhas System; (b) Capinha System; (c) Janeiro Cima System.

It is also concluded that for all systems there was no significant ($p>0.05$) effect of inflow COD concentrations in outflow COD concentrations (R^2= 0.0808 for Aranhas, R^2=0.0089 for Capinha and R^2= 0.2500).

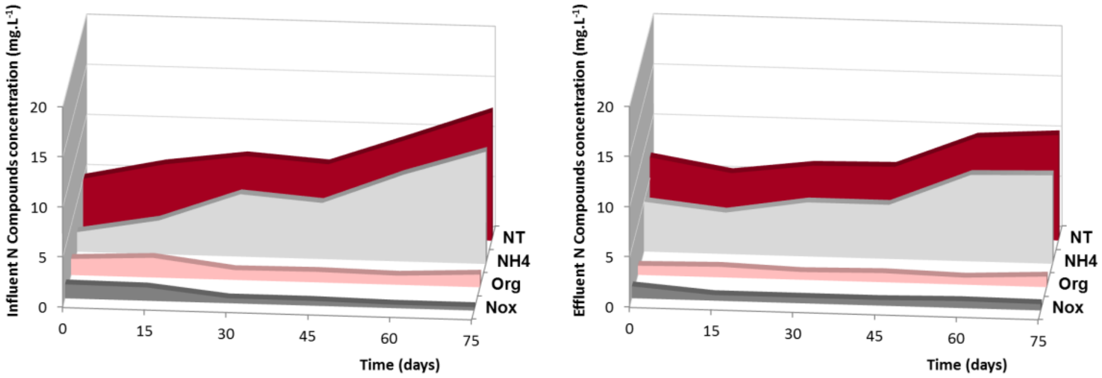
3.3.4. Removal of nitrogenous compounds

The nitrogen cycle at wetland systems is complex and involves several transformations that include ammonia volatilization, ammonification, ammonia sorption to substrata, nitrification, denitrification, assimilation (Armstrong *et al*., 2000). However, according to Kadlec & Knight (1996), the majors mechanisms involved in nitrogen removal in HSSF-CWs are ammonification and nitrification/denitrification, where denitrification is thought to be the mainly nitrogen elimination pathway.

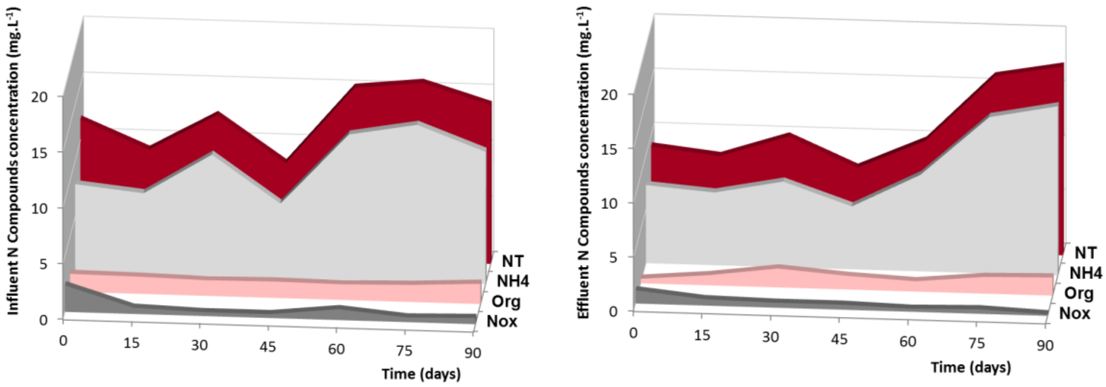
The denitrification consists of the reduction of oxidised forms of nitrogen (NO_x-N) to gaseous nitrogen compounds and therefore, the oxidised nitrogen availability its one of the most important factor that regulates denitrification. As the aerobic conditions are restricted to the rhizosphere and the most part of the HSSF-CWs systems remains in anaerobic or anoxic conditions, the nitrogen removal by denitrification in these systems are probably mainly controlled by the rate of nitrification. As in domestic wastewater the major part of nitrogen is present as ammoniacal nitrogen form, the removal of nitrogen could be very low in these systems. In fact, relatively poor nitrogen removal performance of CWs treating domestic wastewater was often reported in literature (Brix & Arias, 2005; Rousseau *et al.,* 2004). Fountoulakis *et al.* (2009) observed an average removal of TN in HSSF-CWs about 27%. According Vymazal (2007) the magnitude of ammonification and nitrification processes which ultimately remove the TN in HSSF systems is usually low and therefore removal of TN from these systems is commonly low in single-stage CWs.

The systems studied are not different and, as it was expected, the results show that the ammonium-nitrogen is the effectively dominant nitrogen form at inflow of the beds, owing to mineralisation processes in the anaerobic septic and Imhoff tank, representing about ~76% (Aranhas), ~81% (Capinha and ~82% (Janeiro Cima) from total nitrogen, while Org-N representing only 15.9%, 12.3% and 12.1%, respectively. According to Metcalf & Eddy (2003), regarding the levels of TN, Org-N and NH_4^+-N in raw influent (Table 3.1), the domestic wastewater can be classified as a weak concentration domestic wastewater for all systems, since, on average, the values obtained were always lower than 20 mg.L^{-1}, 8 mg.L^{-1} and 12 mg.L^{-1}, respectively. Figure 3.5 also shows the respective distribution of nitrogen forms at the outlet points and the results show that the ammonium-nitrogen remains the predominant form of nitrogen in the effluent at the outlet of all beds studied, representing accounting for more than 80% of total nitrogen in the treated effluent.

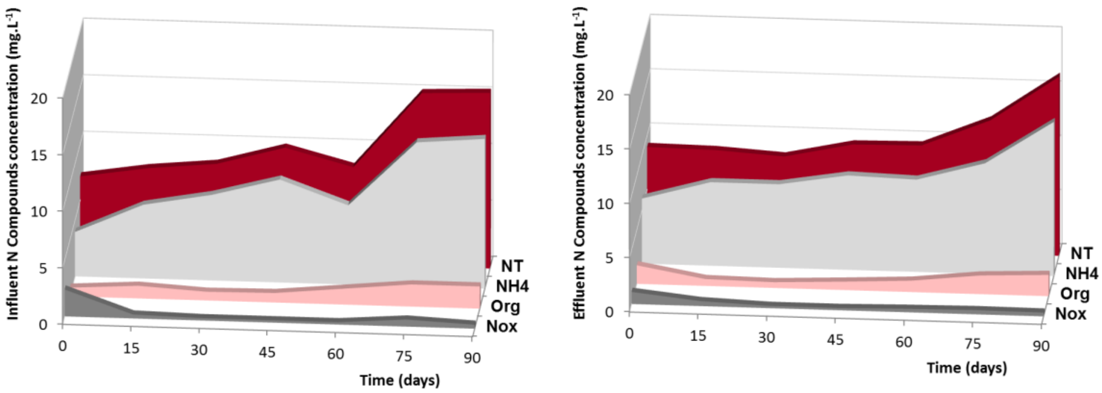
The results for the analyzed nitrogen compounds in the influent also show that the quality at the beds was relative unstable over time, as indicated by the standard deviations of the average values (Table 3.1 and Figure 3.5).



Aranhas



Capinha



Janeiro de Cima

Figure 3.5 Influent and effluent nitrogen fractions during the monitoring period in Aranhas, Capinha and Janeiro Cima Systems.

The systems studied were unable to remove significantly TN and NH_4^+-N levels and even showed relative increases at the outlet of CWs related to inlet, especially on NH_4^+-N levels, during the monitoring period (Figure 3.6 and 3.7). These could be attributed to the decomposition of litter and microbial biomass or ammonification of organic nitrogen. In fact, the ammonification of organic nitrogen could take place, resulting in higher ammonia levels in effluents (Akratos & Tsihrintzis, 2007). The process of ammonification is faster under aerobic conditions, but may also occur under anaerobic conditions and it pH optimal is between 6.5 and 8.5 (Armstrong *et al.,* 2000), approximately about the values observed in this study for all system (Table 3.1).

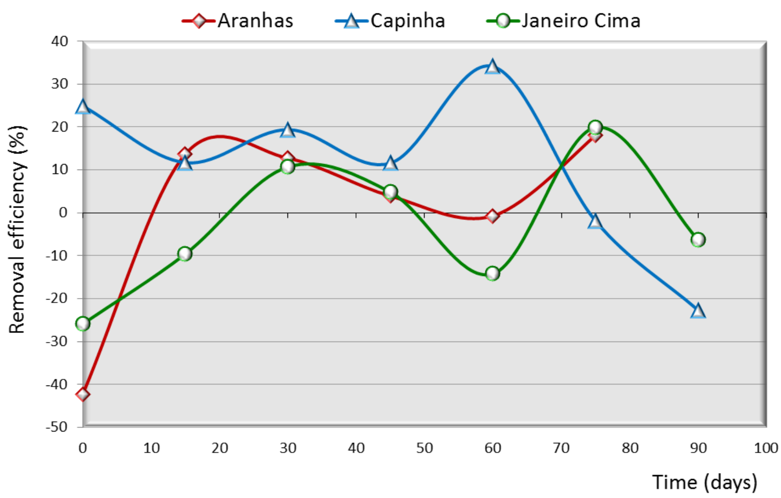


Figure 3.6 Evolution of TN removal efficiency during the study period, expressed in percent of inflow concentrations

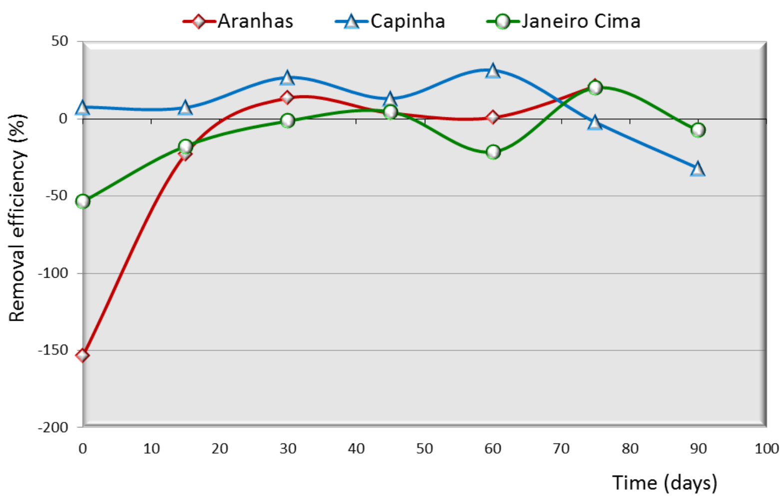


Figure 3.7 Evolution of NH_4^+-N removal efficiency during the study period, expressed in percent of inflow concentrations.

The results show that the mean removal of different nitrogen forms were very low and variable in all systems and for all forms of nitrogen compounds (< 11% for TN, < 30.8% for Org-N, <7% for NH_4^+-N and < 18% for NO_X-N), although the plants were well developed and at the peak of its growth, the HRT (6.6 d (Aranhas), 17 d (Capinha), 9 d (Janeiro Cima) was quite high to promote nitrification and mean wastewater temperature, range between 17 ºC and 23 ºC, was also favourable to the microbial activities related to nitrification and denitrification (Spieles & Mitsch, 2000).

However, according to Vymazal (2005) a unit value of $5m^2$/p.e is necessary to eliminate organic matter and TSS, but it is insufficient to achieve complete nitrification and Kadlec & Knight (1996) show that to obtain significant reductions in nitrogen compounds an unit area values above 12 m^2/p.e. must be used. Since the systems studied have a SSA of 1.5 m^2/p.e. (Aranhas) and 3 m^2/p.e. (Capinha and Janeiro Cima), we may conclude that this may also have been a factor responsible for lower removal rates observed, regard to the different nitrogen compounds analyzed.

Thus, treatment efficiency for different nitrogenous compounds observed in the presen study, in terms of concentration, were quite low compared to the values reported in the literature. Mean removal efficiencies of 42% and 30% for HSSF-CWs were referred for the Czech Republic by Vymazal (2002) and by Brix *et al.* (2007) for Danish wetland system, respectively. Vera *et al.* (2011) also observed that TN removal efficiencies varied between 48 and 66% for full-scale systems in Spain and Puigagut *et al.* (2007) indicated mean efficiencies of 51% for the wetlands evaluated similarly for Spain. Also Albuquerque *et al.* (2008) referred, for a similar system in Portugal, removal efficiency of 76.0%, 78.6% and 71.9% for TN, NH_4^+-N and NO_X–N, respectively. The removal of ammoniacal nitrogen is also significantly lower than those reported in the literature (Korkusuz, 2005; Masi & Martinuzzi, 2007; Vymazal, 2007).

The results obtained showed that the affluent inlet comprised 15.9% (Aranhas), 12.7 % (Capinha) and 12,4 (Janeiro de Cima) of Org-N, 75.6% (Aranhas), 80.9% (Capinha) and 81.9% (Janeiro de Cima) of NH_4^+-N and 8.5% (Aranhas), 6.4% (Capinha) and 5.7% (Janeiro de Cima) of NO_x-N, while the outlet presented 11.5% (Aranhas) 10.7 % (Capinha) and 11,3 (Janeiro de Cima) of Org-N, 79.5% (Aranhas), 84.8% (Capinha) and 84.9% (Janeiro de Cima) of NH_4^+-N and 9.0 % (Aranhas), 4.5% (Capinha) and 3.8 % (Janeiro de Cima) of NO_x-N, i.e. very similar proportions. However, there was an enrichment of the soluble nitrogen species (NH_4^+-N and NO_x-N) through the beds which may be indicative of the processes of nitrification/denitrification.

A great variability in the Org-N removal was observed along the monitored period. These fluctuations were probably a result of the low inflow concentrations (from 0.91 to 1.96 $mg.L^{-1}$ for Aranhas, 1.40 to 1.82 $mg.L^{-1}$ for Capinha and 0.77 to 1.96 $mg.L^{-1}$ for Janeiro Cima), because in these conditions the internal production and the release of nitrogen may be greater than assimilation, resulting in negative removal efficiencies (Kadlec & Knight, 1996), as we have observed occasionally over the sampling period. The reduction in the Org-N could be attributed to biologically reactions of assimilation and ammonification in the wetlands which commonly result in increases in the NH_4^+-N values of the substrate and decreases in organic values fraction.

Oxidized nitrogen removal (mainly nitrates) was generally similar in all wetlands and no important differences between inlet and outlet of the CWs were observed (Table 3.1). However, in some instances it was observed an increase of NO_X-N concentrations within HSSF-CWs effluent compared to the inlet. These results could indicate that or had no removal or removal efficiency was very low, but also indicated that NO_x-N formed by nitrification of ammonium presented in influent wastewater are not accumulated in the wetlands and processes like denitrification could occurred, despite the results indicate poor denitrifying activity in the beds.

As Vymazal & Kröpfelová (2008) indicated that there is a good linear relationship between inflow and outflow concentrations for both TN and NH_4^+-N, we could referred that the lower removal efficiencies observed for these analysed forms of nitrogen may be due to the very lower inlet concentrations (4.97 to 16.03 $mg.L^{-1}$ for TN and 1.96 to 14.07 $mg.L^{-1}$ for NH_4^+-N). However, the lack of oxygen available for nitrification was probably the main factor that limited the process of nitrogen removal since ammonium is the dominant species in the influent at CWs studied and the limited oxygen transfer capability of these systems could be explained the low efficiency of ammonium conversion to NO_x-N in wetland systems. On the other hand, the high values of organic compounds present in inflowing wastewater at CWs studied (Table 3.1) and their respective decay through aerobic heterotrophic bacteria, which could have competed with nitrifying bacteria for oxygen available, could also contribute to explain the lower removal efficiencies obtained at the present study.

Kouki *et al.* (2009) reported negative correlations between BOD_5 rates and dissolved oxygen (DO) concentrations and Total Kjeldahl Nitrogen (T_KN) amounts, indicates that the decrease of these two pollutants is dependent on the oxygen availability. The same authors obtained a positive correlation (r=0.65) between effluent BOD_5 and T_KN concentrations, what shows that residual nitrogen loads becomes greater when the consumption of oxygen is increased mainly through biodegradation processes. Therefore, at very high organic concentrations, as occurs in the systems studied (Table 3.1), must of the available oxygen that is transported into the macrophytes roots will be used for the removal of the biodegradable organic matter and this maybe inhibits the establishment of a large population of nitrifying bacteria . Also El Hamouri *et al.* (2007) confirmed the general opinion that HSSF-CWs systems are not efficient for N removal since the amount of oxygen made available by the roots to the nitrifying bacteria is far from being able to support both carbon and nitrogen oxidation processes. Also Riley *et al.* (2005) observed that ammonia removal tend to decreased with an increase in influent COD.

The optimum pH value for the nitrification process is around 7.6, (Arendacz *et al.,* 2008), therefore lower values observed in the beds (Table 3.1) throughout the study could also explain the low NH_4^+-N removal efficiency.

During the sampling period, the wastewater entered the systems with an average values of TN and NH_4^+-N loads of 0.63 $gm^{-2}.d^{-1}$ and 0.47 $gm^{-2}.d^{-1}$ (Aranhas), 0.36 $gm^{-2}.d^{-1}$ and 0.29 $gm^{-2}.d^{-1}$ (Capinha) and 0.57 $gm^{-2}.d^{-1}$ and 0.47 $gm^{-2}.d^{-1}$ (Janeiro de Cima), respectively. The NO_x-N inflow load had very low values (under 0.06 $gm^{-2}.d^{-1}$). The concentrations of TN in the effluents are correlated to its loadings rates and these relationships are shown in figure 3.8 for all beds. Linear regression showed statistically significant ($p<0.05$) increase in outlet TN concentrations with increasing TN mass loading rate, since high coefficients of determination (R^2 ranged from 0.5463 to 0.8932) were observed and significant correlation was also seen between the two variables with a *p-value* less than 0.05 (p= 0.000; r=0.859 for Aranhas), (p= 0.000; r=0.739 for Capinha) (p= 0.000; r=0.890 for Janeiro Cima).

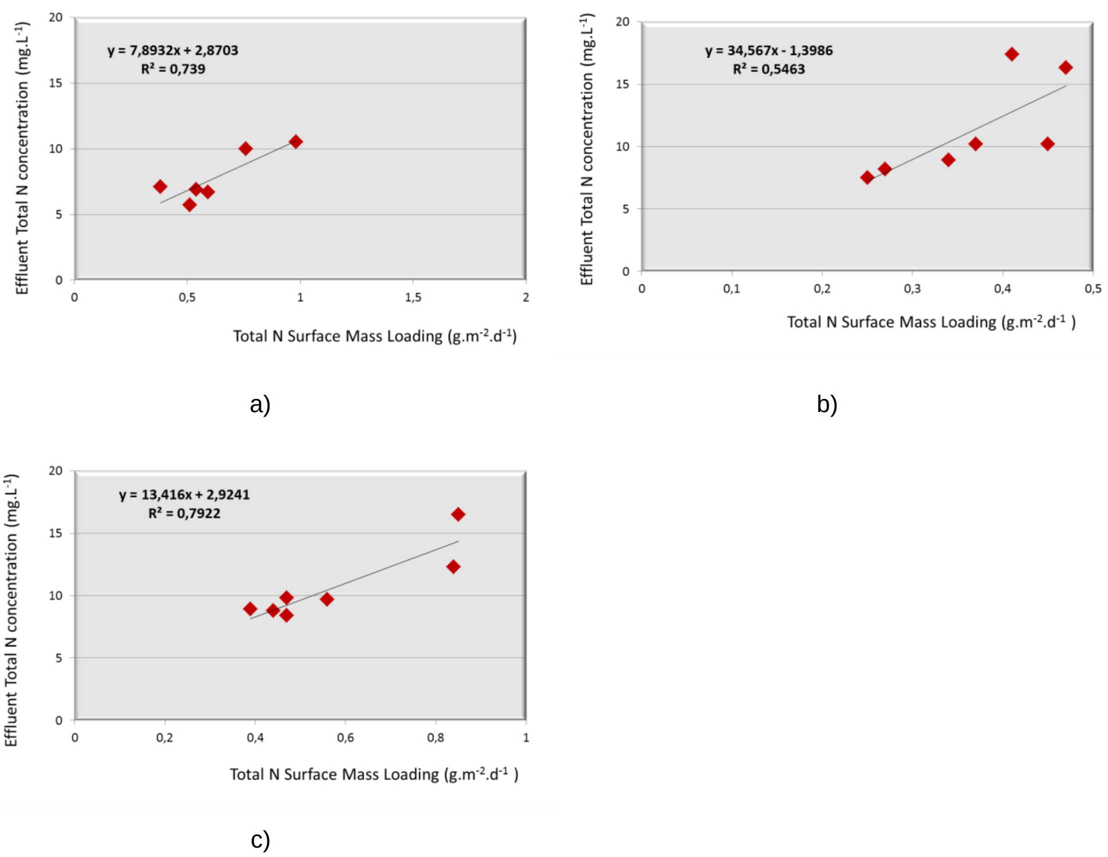


Figure 3.8 Relationship between effluent TN concentrations and TN mass loading: (a) Aranhas System; (b) Capinha System; (c) Janeiro Cima System.

Similar results were seen for NH_4^+-N effluent concentrations and its inlet loads. However, only for Aranhas and Janeiro Cima we found that there are a significant ($p<0.05$) linear proportional relationship between those two variables with high coefficient of determination of 0.7926 and 0.7979, respectively, while for Capinha system the relationship was not obvious (R^2= 0.0318), suggesting there was no significant ($p>0.05$) effect of loading rates on NH_4^+-N concentration in the effluent of this wetland. In fact, the correlations of these two variables showed a p and r values of 0.000 and 0.0890 (Aranhas), 0.000 and 0.893 (Janeiro Cima) and 0.003 and 0.178 (Capinha), showed that for Capinha the correlation are not significant.

For TN and NH_4^+-N it has been observed that there was no significant effect of their mass loading rate on their RE and apparently these two variables had no significant correlation with *p-value* more than 0.05.

Considering the general performances of all systems, it can be notice that the outlet concentrations of TN have generally satisfied the national limits for discharging in superficial water for facilities less than 2 000 P.E. (15 $mg.L^{-1}$) and usually, the final effluent also complies with the Directive 271/91/EEC guidelines for TN (15 $mg.L^{-1}$) likely because of low inlet concentrations. For NH_4^+-N concentration the results showed that, generally, the values are lower than that required by discharge standards in Portugal for p.e. < 2000 inhabitants (< 10 $mg.L^{-1}$). Mean concentrations of nitrogen oxides (nitrite plus nitrate) in the outlet effluent (0.70 $mg.L^{-1}$, 0.51 $mg.L^{-1}$ and 0.44 $mg.L^{-1}$ for Aranhas, Capinha and Janeiro Cima, respectively) of the CWs was significantly lower than those in the influent (0.74 $mg.L^{-1}$, 0.80 $mg.L^{-1}$ and 0.62 $mg.L^{-1}$ for Aranhas, Capinha and Janeiro Cima, respectively).

3.3.5. Removal of phosphorus compound

In generally, the HSSF-CWs tend to do not remove higher amounts of phosphorus because the materials used in substrata are poor in iron (Fe) and aluminium (Al) hydrous oxides minerals as well as in calcium (Ca) and magnesium (Mg) concentrations, elements essentials for adsorption and precipitation of insoluble forms of phosphorus in wetlands, the most important mechanisms for removal phosphorus in these systems (Kadlec & Knight, 1996). The uptake capacity of macrophytes seems limited and so, are relatively less important on phosphorus removal (Brix, 1994). However, Tanner (2001) referred that for low TP concentration in the wastewater, absorption by plants and assimilation by the microorganisms can become important for the TP removal.

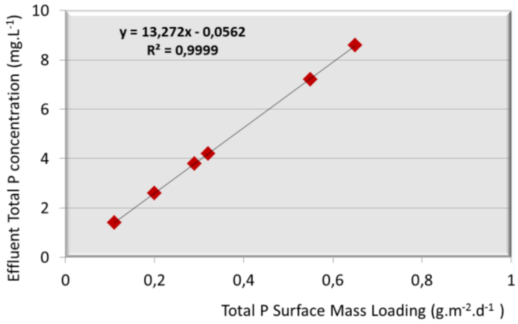
The inlet concentration of TP varied between 1.44 and 8.57 $mg.L^{-1}$ (Aranhas), 1.55 and 9.73 $mg.L^{-1}$ (Capinha) and 2.64 and 8.95 $mg.L^{-1}$ (Janeiro Cima) and therefore can be classified as weak to moderate phosphorus load the wastewater affluent to all systems (Metcalf & Eddy, 2003). The outflow concentration had a significant ($p<0.05$) reduction in comparison with the inflow concentrations levels and varied between 0.19 and 2.83 $mg.L^{-1}$ (Aranhas), 0.50 and 4.7 $mg.L^{-1}$ (Capinha) and 0.9 and 2.9 $mg.L^{-1}$ (Janeiro Cima). The inflow/outflow average TP concentrations were 4.65/1.89 (Aranhas), 6.25/3.10 (Capinha) and 6.52/2.10 (Janeiro de Cima) (Table 3.1). The linear relationship between inflow and outflow TP concentrations is poor (R^2=0.1904) and apparently there was no significant ($p>0.05$) effect of TP influent concentrations on TP effluent concentrations.

The RE of TP in terms of concentrations was 44.3% (Aranhas), 41.2% (Capinha) and 65% (Janeiro Cima). These results are in accordance with previous studies observing removal efficiencies of 25-65% (Chen *et al.,* 2006; Tsihrintzis *et al*., 2007) and are consistent with others studies published for full-scale wetlands (Vymazal, 2002, Rousseau *et al.,* 2004, Brix *et al.,* 2007; Vera *et al.,* 2011). The fact that pH of the influent remain roughly stable through the beds could also have contributed to the stability of precipitated phosphorus compounds within in systems.

However, in some cases phosphorus output was higher than input probably due to the release or de-sorption of some phosphorus. A possible mechanism that may be involved in P release in these wetlands is the lack of oxygen that resulting in anaerobiosis that increases P availability, because under such reductions conditions, iron and other hydrous oxides minerals can be converted into soluble forms causing solubilisation and release of bound P into the water column (Rousseau *et al*., 2004; Vymazal, 2007; Vera *et al.,* 2011). This might be also attributed to intermittent release of P back into the system from accumulated unharvested biomass at the bed's surface after macrophytes decay. As, in Portugal, the majority of media utilized in the HSSF-CWs is gravel that have a low retention capacity, the TP removal efficiency could not be enhanced greatly.

HSSF-CW is generally thought to have a greater potential to remove nitrogen than phosphorus (Vymazal *et al*., 1998). It was therefore surprising to find removal of TP in CWs systems studied to be significantly higher than the corresponding TN removal. Assuming adsorption is partially responsible for phosphorus fixation, it appears that the adsorption capacity of the bed media does not show as yet to have been expended after the 48 months of operation.

The total phosphorus loading rates in the systems investigated ranged from 0.11-0.65 $g.m^{-2}.d^{-1}$ (Aranhas), 0.05-0.28 $g.m^{-2}.d^{-1}$ (Capinha) and 0.14-0.49 $g.m^{-2}.d^{-1}$ (Janeiro Cima). Figure 3.9 illustrates an increase of effluent TP when increasing inlet load, but the regression coefficient indicates that only for Aranhas system there were a clear and strong relationship between the effluent concentrations of TP and its mass loading rate, with statistical analysis demonstrated that 99.9% of the total variation in TP effluent concentration (dependent variable) could be explained by the TP mass loading rate (independent variable). These two variables also had a significant correlation ($p=0.004$; $r=0.999$) in this wetland.



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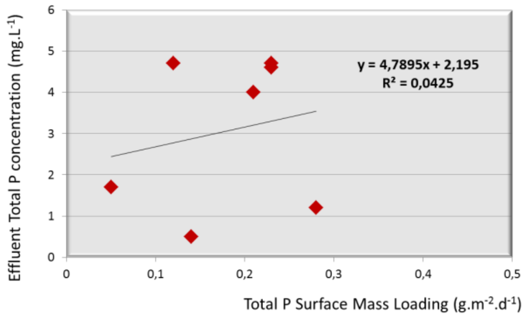
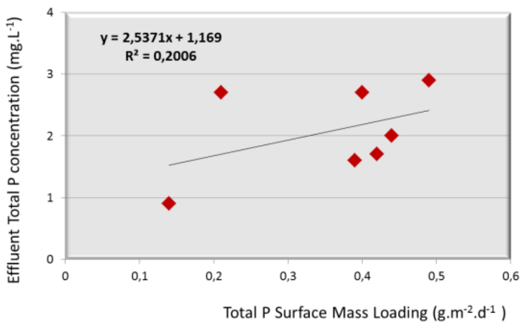


b)



c)

Figure 3.9 Relationship between effluent TP concentration and TP mass loading rate: (a) Aranhas System; (b) Capinha System; (c) Janeiro Cima System.

After treatment, the effluent concentrations always met the established like limit (10 mg.L^{-1}) by discharge standards in Portugal for WWTP from Population Equivalent < 2000 inhabitants in freshwaters. However, the results obtained evidenced that the concentration of TP in the treated effluent does not always meet the limit established for discharge into waters that feed reservoirs (3 mg.L^{-1}, according to the Decree Law No. 236/98 of 1 August), or the limit set in the Urban Wastewater Directive (271/91/EEC) for sensitive zones, which is 2 mg.L^{-1}. In fact, this threshold has been met only in 33% of samples to the system of Aranhas and 43% of the samples in the case of the other two systems.

3.4. Conclusions

The evaluation of performance of three CWs with horizontal subsurface flow in the Central Region of Portugal at summer period revealed that these systems provide sustainable high values of removal for organics and TSS in the study period and the per-treatment installed seems facilitated the function of the systems by preventing clogging, odour, flies nuisance, besides adding to system efficacy. However, influent and effluent TSS concentrations in the systems examined are generally higher that the reported for other European systems, despite the fact that the removal efficiency of TSS fits pretty well with the reports consulted literature. Generally, the results show that both the organic and the solids loads had a high variation during the summer period, whilst the influent loads of nitrogen and phosphorous were low. The variations in COD and TSS removal efficiency were probably the result of differences in COD and TSS concentrations and load inflowing among the period study.

Despite to the unit area for the studied wetlands was below the recommended design value of 5 m^2/p.e. for this type of wetlands, the use of a primary treatment system (septic and Imhoff tank) combined with a HSSF system allowed to ensure the total average TSS and COD removal efficiencies > 76.8 % and > 58.7%, respectively and all the results obtained with respect to the TSS removal efficiencies are consistent with evaluations for full-scale HSSF-CWs systems. The COD removal efficiency observed for the systems studies seems to be lower than ones found in similar systems in Mediterranean countries and other regions of Europe, which could be associated to the discharge of influents from small agro-industries that increased the particulate hard-to-biodegraded organics.

The mean phosphorus removal efficiencies were 59.4%, 50.4% and 67.8% for Aranhas, Capinha and Janeiro Cima systems, respectively. In comparison with value reported in literature and especially for Europe (47.1%), the average removal efficiencies of TP were general higher. As TP removal being dependent on retention times (Tanner *et al*., 1999), the higher TP removal in systems studies may be attributed to higher evapotranspiration rates, which leads to higher retention times in summer. However, removal of nitrogenous compounds was much lower than expected and than that referred in literature (an average TN and NH_4^+-N removal of 39.6% and 30%, respectively). In fact, nitrogen compounds were the least efficiently removed pollutant with an overall percent reduction of only <11% (TN), <31% (Org-N), <7% (NH_4^+-N) and <18% (NOx-N). This circumstance may be associated to the entrance of low concentration of TN (<16 $mg.L^{-1}$) and NH_4^+-N (<13 $mg.L^{-1}$), although the all beds were well developed and the mean HRT was over 6.6 d.

Despite the removal of nitrogen compounds is low, but due to the low inflow concentration, the outflow concentrations are also low. As the process of nitrification-denitrification is recognized as the primary process responsible for the removal of nitrogen in HSSF-CWs, we can be concluded that the nitrogen removal process is low due to the amount of oxygen present in these systems and exacerbated by the fact that such low oxygen content is still disputed between the processes of nitrification and decomposition of organic matter. The low removal rate of NH_4^+-N showed that nitrification was not very active and apparently, the major reason for the low ammonia removal was probably the inability of systems to nitrify. In this study, the wastewater pH in the CWs was always < 7.2, therefore it was assumed that the contribution of ammonia volatilization in the nitrogen output of these systems were low or negligible. Increase of inorganic nitrogen in the effluent was detected and occasional negative values were obtained for the removal rate in all systems for all nitrogen fractions.

Aranhas system was in general less efficient, particularly for COD and TSS due to the usually higher average loading rate of these parameters applied to this bed, which is also mainly associated with the lowest per unit area equivalent populational.

A significant ($p<0.05$) relationship between mass load and removal efficiency on a percentage basis was observed only for TSS and COD suggest that the HSSF-CWs are very robust for wastewater treatment that has shown a good tolerance to fluctuations on influent concentrations and loading rate. This is may be explained by the long 6,6 days, 17 days and 9 days HRT for Aranhas, Capinha and Janeiro Cima, respectively, which creates a high buffering capacity to cope with a highly variable nature of the incoming wastewater, in agreement with findings of Kadlec & Knight (1996).

In generally, for all pollutants the effluent concentrations increase as mass loading rate increases. However, the results obtained suggest that HSSF-CWs can contribute to reduce effectively the TSS and COD presented in wastewater to levels that are in according to the Portugal regulatory standards effluents levels.

Phosphorus and nitrogen discharge in water bodies are critically and the main cause of eutrophication and the discharge of domestic wastewater is one of the main inputs of these elements into natural waters. So, it is important to treat the wastewater for the removal of these nutrients to levels that are acceptable for natural systems especially freshwater ecosystems. Although the systems were not removed significant nitrogen compounds, concentrations of total and ammonium nitrogen in the produced effluent were generally lower than that required by discharge standards in Portugal for WWTP from population equivalent < 2000 inhabitants, which are ≤ 15 $mg.L^{-1}$ and ≤ 10 $mg.L^{-1}$, respectively.

The HSSF-CWs seems to be a good alternative to the conventional systems especially in rural areas, where they can also encourage the reuse of treated wastewater in agriculture, often dominant activity in these zones, as is the case of the region studied. The reuse of wastewater treated is very important given the scarcity of water in the world. As The Portugal is one of the countries where desertification has particular relevance, with about 60% of the territory susceptible to desertification and drought, as a result of our climatic, geological and vegetation cover, we understand that such situation determine the necessity and importance of implementing measures that contribute to an appropriate sustainable management of water resources in our country. So, the reuse in agricultural of treated effluent could be one of those measures, which could provide a source of water and nutrients for this activity, which has particular expression in the studied region.

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4. Influence of climate and seasonality in pollutant removal by a full-scale HSSF-CW in a continental-Mediterranean region of Portugal

Presented in [2]5th International Symposium on Wetland Pollutant Dynamics and Control (WETPOL 2013), 13 -17 October 2013, Nantes, France (poster, published in CD Rom) and in [3]Symposium of the FibEnTech Research Unit: Fiber Materials and Environmental Technologies Faculty of Engineering, 28-29 January 2016, Covilhã, Portugal (oral presentation, published in CD Rom).

Abstract

Eleven months monitoring program was setup in a horizontal subsurface flow constructed wetland (HSSF-CW) treating domestic wastewater at Sarnadas (Rodão, Portugal) for evaluating the influence of climatic conditions (Continental-Mediterranean Climate region) and season characteristics (spring-summer and autumn-winter periods) on the removal of pollutants. Average BOD_5 removal was above 88%, showing no significant changes between the two seasonal periods, whilst CODt and CODs increased by 4.3% and 6.2%, respectively, during spring-summer period, although this increase was not statistically ($p>0.05$) significant. No significant linear correlation was observed between effluent TSS, CODt and CODs concentrations and the respective mass loading rates. A positive linear ($p<0.05$) central tendency regression was observed between effluent BOD_5 concentrations and BOD_5 loading rate. A linear relationship between hydraulic loading rate (HLR) and removal efficiency (RE) was considered statistically ($p<0.05$) significant for BOD_5, but only for the spring-summer period. Nitrogen and phosphorus removal were relatively low, but the results allow concluding that season had a significant ($p<0.05$) effect on the reduction of TN, NH_4^+-N, NOx-N, TP and DP, with higher values in spring-summer period (10.4%, 10.4%, 3.4%, 27.5% and 26.1%, respectively). No significant linear relationship was observed between HLR and RE for N and P compounds for any season period. TN and NH_4^+-N concentrations in the effluent tend decreased significantly ($p<0.05$) with the increase of the

[2] Mesquita, M.C.; Carreiro, F.; Albuquerque, A., Amaral, L.; Nogueira, R. (2013). "Seasonal Performance of a full-scale constructed wetland system for Sarnadas de Rodão (Portugal) domestic wastewater treatment". (published in CD-Rom)". Poster presentation in 5th International Symposium on Wetland Pollutant Dynamics and Control (WETPOL 2013). 13 - 17 October 2013, Nantes, France

[3] Mesquita, M.C; Albuquerque, A; Amaral, L.; Nogueira, R. 2016. "Phosphorus removal from domestic wastewater in horizontal subsurface flow constructed wetland with *Phragmites australis*". Oral presentation in 1st Symposium of the FibEnTech Research Unit: Fiber Materials and Environmental Technologies Faculty of Engineering, 28-29 January, Covilhã, Portugal.

incoming mass load rates for whole monitoring period and during spring-summer period. Results showed that HSSF-CWs is a suitable solution for the removal of TSS, COD and BOD and its efficiency is not significantly affected by changes in season characteristics.

Keywords: Horizontal subsurface flow, Constructed wetlands, Domestic wastewater, Continental-Mediterranean climate, Phragmites australis, Removal efficiency, Seasonal characteristics.

4.1. Introduction

The increasing production of wastewater as a result of human settlement, population growth and agricultural and industrial activities has been contributing for the pollution of fresh water bodies. However, the increasing scarcity of water resources in the world along with the high rate of urbanization has led to the need of implementing best water resources management practices based on principles of sustainability and on recovery and reuse of wastewater mainly for irrigation (Masi & Martinuzzi, 2007; Marecos do Monte & Albuquerque, 2010).

Recent legislation such as the Water Framework Directive (WFD, Directive 2000/60/EEC of 23 October) established the bases and the institutional framework for sustainable water management and has become increasingly stringent in terms of water quality standards for guarantying the quality of the receiving waters for different uses. The treatment of municipal wastewater has been adjusted to fulfill the WFD and, for small towns, ecological technologic solutions, such as constructed wetlands (CWs), have been developing for that purpose.

The most commonly used technologies for municipal wastewater treatment in Portugal are the conventional activated-sludge process and trickling filters systems. However, for small communities (i.e. < 2000 inhabitants), low cost and ecological solutions, based on CWs technology, has become very popular in many countries of the world and many studies have shown the overall effectiveness of these systems in treating wastewater (Cui *et al*., 2015; Rai *et al*., 2015; Zhang *et al*., 2015). In Mediterranean countries there are also reports of the successful application of these systems, particularly in municipal and domestic sewage treatment, as secondary and tertiary stage, after a pre-treatment and a primary sedimentation stage on a septic tank or Imhoff tank (El-Hamouri *et al*., 2007; Puigagut *et al*., 2007; Albuquerque *et al.,* 2009; El-Refaie, 2010; Garfi *et al*., 2012; Toscano *et al*., 2015).

The benefits described for these self-sustainable systems include low costs associated with maintenance, operation and energy requirements; good treatment performance resulting in a overall decrease of risk to the environment and public health associated to the discharge of untreated or inappropriately treated wastewater (Scholz *et al*., 2010; Dan *et al.*, 2011;). At the same time, these systems seem to be more flexible than conventional treatment systems and therefore showing good capacity to adapt to flow and pollutant load fluctuations (Kadlec & Wallace, 2009; Brix *et al*., 2007), characteristics of wastewaters from small villages that tend to be more fluctuating and are generally more concentrated than those from urban areas (Metcalf & Eddy, 2003). In Portugal, the most commonly used are the constructed wetlands with horizontal subsurface flow (HSSF-CWs) (Albuquerque *et al*., 2009; Galvão, 2009; Duarte *et al.*, 2010).

Due to usually long retention times, the HSSF-CWs can provide a reliable secondary level of treatment showing that they are effective in removing total suspended solids (TSS), and organic matter (BOD and COD) and also removing nutrients such as nitrogen (N) (Vymazal, 2007; Kadlec & Wallace, 2009; Lee *et al*., 2009; Mander & Mitsch, 2009; Ko *et al*., 2012).

Several studies have been performed and the removal efficiencies (RE) of pollutants varied between 72% to 95% for TSS, 71.2%-94.1% for BOD, 59.7% to 89% for COD and 21% to 56% for N (Headley *et al*, 2005; El-Hamouri *et al.*, 2007; Tuszynska & Obarska-Pempkowiak, 2008; Vymazal & Kröpfelová, 2008). Regarding to phosphorous (P) removal it was reported values between 30% and 60%, depending on the wastewater and the media used (Vymazal, 2007; Cui *et al*., 2008). Little information exists on the performance of these systems in Portugal for depending on seasonal characteristics (i.e. temperature, evapotranspiration and rain changes). However, Albuquerque *et al.* (2009) conducted one of the first studies of the performance of CWs in Central region of Portugal and reported RE of 66.7%, 56.4% and 76%, for COD, TSS and TN, respectively.

Also, Galvão (2009) developed field studies with data analysis collected during an extended period of time, concerning the hydraulic and environmental performance of two full-scale CWs in operation in south region of Portugal and reported the following average removals rates: BOD: 64-74%; COD: 50-58% and TSS: 71-80%. Oliveira (2007) carried out a study in four CWs systems in the north of Portugal and verified that the RE were from 78% to 85% for COS, 84% to 96% for BOD_5, 60% and 94% for TSS and 4% and 51% for ammonia.

Pollutant RE in CWs are affected by numerous factors, such as hydraulic retention time (HRT), hydraulic loading rate (HLR), pollutant concentrations, mass loading rates, wetland design, substrate characteristics, vegetation type, presence or absence of plants, or a combination of these parameters (Kadlec & Knight, 1996; Wallace & Knight, 2006; Rousseau *et al.*, 2008; Taylor *et al*., 2011; Bialowiec *et al*., 2014). Several studies also have shown that climatic conditions and seasonality may affect contaminant removal efficiency, especially nitrogen removal (Kadlec & Wallace, 2009; Garfi *et al*., 2012; Hijosa-Valsero *et al*., 2012).

During rainy season excessive precipitation may considerably alter water levels in these systems, resulting in increased flow velocities and reduced contact times, cause contaminant dilution and modification in the water chemistry (Katsenovich *et al*., 2009). Especially under warm conditions, changes in volumetric flow attributed to evapotranspiration can contributed to change the treatment performance by removing water from the system (thus increasing HRT) and increasing concentrations of the effluent pollutant concentrations (Kyambadde *et al*., 2005; Morari & Giardini, 2009). Chazarenc *et al.* (2010) observed that higher ET was beneficial to total Kjeldahl nitrogen (T_KN) removal and attributed this fact to an increase the HRT. Also Katsenovich *et al.* (2009) reported higher removal of total dissolved P during dry season, despite they noted higher outflow concentrations due to the high evapotranspiration rate.

The rate of mineralization of organic nitrogen to ammonia seems to double with a temperature increase of 10 ºC and the optimum temperature for this reaction was between 40 to 60 ºC (Reddy & Patrick, 1984 cit. by Vymazal, 2007). For a good nitrification, the optimal temperature is between 30-40 ºC (Kadlec & Wallace, 2009). Akratos & Tsihrintzis (2007) conducted a study investigating a wide range of temperatures (2 to 26°C) and they concluded that a HRT greater than eight days (loading rate < 35 $kgBOD.ha^{-1}.day^{-1}$) is recommended for at least a 90% reduction in BOD and was also sufficient to achieve high ammonia removal at temperatures greater than 15°C, but a residence time of at least 20 days was needed to achieve about 70% ammonia removal at temperatures less than 15°C.

Sirivedhin & Gray (2006) showed that denitrification rates varied from 0.0021 to 0.810 $kgN.kg^{-1}$ sediment per day as temperature increased from 4 to 25 ºC. However, it is not clear if the lowest efficiency observed during the winter period is due to the lower temperatures alone or is the combined effect with increased hydraulic loadings, because several other studies have not shown significant treatment differences between winter and summer (Neralla *et al*., 2000; Vymazal, 2001; Maehlum & Jessen, 2003; Steinmann *et al*., 2003; Yang *et al*., 2007; Dzakpasu *et al*., 2011).

Song *et al* (2006) reported that BOD_5, COD, ammonia-nitrogen (NH_4^+-N), and total phosphorus (TP) removal efficiencies displayed seasonal variations, with removal rates of BOD_5 and COD to be more efficient in spring and summer than in autumn and winter, while NH_4^+-N and TP removal were more efficient in summer and autumn than in spring and winter. Also Jing & Lin (2004) observed that season variation affected the performance of a CW in removing NH_4^+-N from polluted river water in Southern Taiwan and that removal efficiency not only varied cyclically with the seasons, but also increased exponentially with water temperature. Albuquerque *et al*., (2009) obtained different COD removals between spring and summer with average values of 57.8% and 60%, respectively, at the Central region of Portugal. However, they did not found any differences in those two periods for the removal of TSS. For nitrate (NO_3^--N) and total nitrogen (TN) removal, they observed a tendency for an improvement in the RE for the summer period.

Generally, organic and N removal seems to be more strong influenced by climatic and season factors than that of TSS and P removal because the organic and N processes are mainly associated to microbial activity, while TSS and P removal are more influenced by physical and/or chemical processes such as sedimentation, precipitation and/or sorption to the medium filling that are not as sensitive to temperature effects (Spieles & Mitsch, 2000). Nevertheless, seasonal differences can affect P removal due to higher uptake by plants during the growth season and lower at senescence period, although it is generally referred that there are less variance in season P removal when compared to N (Picard *et al.*, 2005; Vymazal, 2007).

Although considerable numbers of studies have contributed to understanding the mechanisms associated to removal process in these systems, inconsistence in the results suggest the importance of further studies to contribute for the optimization of this technology and thus ensuring that they comply with the increasingly strict requirements discharge standards for treated effluents and for its reuse. On the other hand, although the effects of climate and seasonality are considered relevant for the performance of the treatment of CWs, its role remains unclear (Dong *et al*., 2011; Garfi *et al*., 2012; Mancilla-Villalobos *et al*., 2013; Sharma *et al*., 2013) and at regions with Mediterranean climate daily temperature fluctuation are higher than regions with cold or tropical climates, which mainly have been carried out the studies on these systems (Vymazal, 2011).

Therefore, the aim of this study was to evaluate the performance of a full-scale HSSF-CW located in a Centre Interior region of Portugal under the effect of different seasonal and climatic conditions.

4.2. Materials and methods

4.2.1. Wastewater treatment System description

The study was carried out on a full-scale wastewater treatment plant (WWTP) in Sarnadas Rodão, a small village of 637 inhabitants (INE, 2012), in the Centre Interior region of Portugal (39° 45' 32" N 7° 38' 35" W) (Figure. 4.1). The region is in the warm temperate zone, corresponding to a Mediterranean climate, clearly influenced by continentally (IPMA, 2013a)**.** The climate is characterized by cold winters alternating with hot and dry summers. The two warmest months, July and August, have temperatures above 24 ºC on average, while winters are harsh, with the months of December, January, February, showing average temperatures below 10 °C. The difference between the warmest month (July) and coldest month (January) is 16.2 ºC. Average annual temperature is 15.6 °C, with average minimum and maximum temperatures of 11 and 20ºC, respectively. There are episodic heavy rains, contrasting with a total rainfall rather moderate, and approximately 50% of the average annual rainfall occurs between November and February, while July, August and September are usually the driest months. The average annual precipitation for the period under review (1961-1986) is 780.7 mm, ranging between 500 and 900 mm (IPMA, 2013a and 2013b).

The WWTP is operating since 2006 and was projected for treating the domestic wastewater produced by a population of 550 equivalent inhabitants, with an average flow rate of 60 $m^3.d^{-1}$ (2006) and 73 $m^3.d^{-1}$ at 2020. At present, the population is 637 inhabitants, i.e. is greater than the value considered for sizing the beds. The treatment system consists of pre-treatment with a single manually cleaned screen bars and a sand channel followed by a primary treatment in a three compartmentalized septic tank. The septic tank provides 2 days of HRT in order to ensure sedimentation of part of the suspended solids and separate oils and grease presents in the raw influent and perform an equalization of the wastewater, ensure a constant flow entering in the wetland system and prevent it clogging. As secondary level of treatment, there is a HSSF-CW with 40m x 28m (length and width), with a total surface area of 1120 m^2, which corresponds to an area per equivalent inhabitant of about 2 m^2, which is lower than the range of values using simple "rule of thumb" 3 to 6 m^2/equivalent-inhabitant recommended for HSSF-CWs systems (Rousseau *et al*., 2004; Vymazal, 2007; Kadlec & Wallace, 2009).

The media bed is composed by gravel (40 cm at the bottom), sand (10 cm at the medium) and topsoil (10 cm), planted with common reed plants (*Phragmites australis*), and was designed for operated at a HLR between 14 cm d^{-1} and 17 cm d^{-1}, which falls within the values (2-20 cm d^{-1}) referred to HSSF-CWs systems (IWA, 2000; Vymazal & Kröpfelová, 2008). The medium porosity of filter media is of 0.38. The bed was sized for having a maximum organic loading rate (OLR) of approximately 10 $gBOD_5.m^{-2}.d^{-1}$, TSS loading rate of 22 gTSS. m^{-2}. d^{-1}, a total N loading rate (NLR) of 2.2 gTN. m^{-2}. d^{-1}, while it is expected a total P loading rate of (PLR) 0.44 gTP. m^{-2} d^{-1} and for dealing with HRT between 3.5 and 4.3 days.

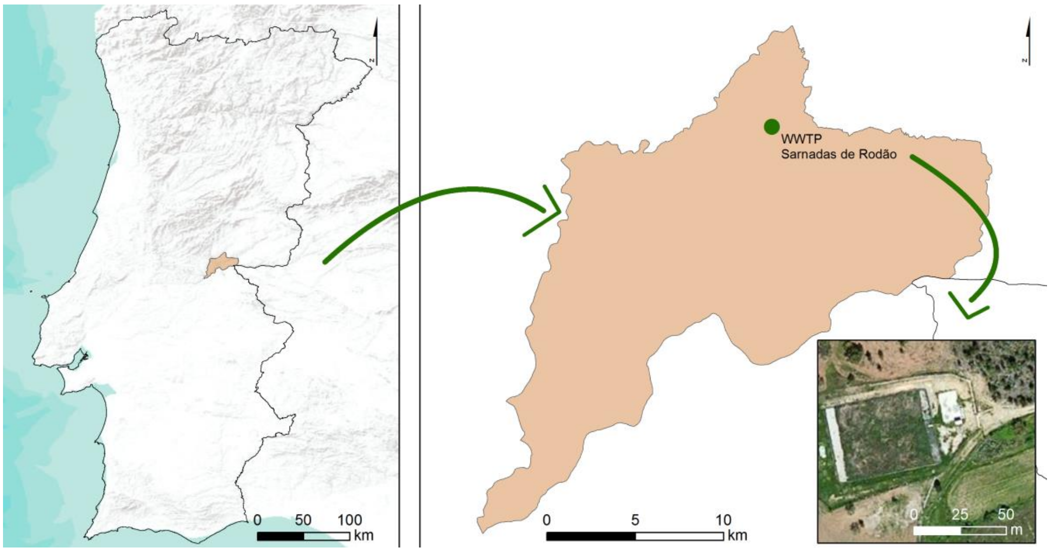


Figure 4.1 Location site of the Sarnadas Rodão WWTP (Portugal)

The WWTP should guarantee a treatment that meets the standards of TSS ≤ 60 $mg.L^{-1}$, COD ≤ 125 $mg.L^{-1}$, BOD5 ≤ 25 $mg.L^{-1}$, TN ≤ 15 $mg.L^{-1}$ and TP ≤ 2 $mg.L^{-1}$ (Portuguese Decrees-Law No. 152/97 and 236/98).

4.2.2. Sampling and analysis

The sampling took place monthly from August 2012 to June 2013 in order to evaluate wetland performance under different climatic conditions, at inlet of the HSSF (after septic tank) and at the outlet of the wetland bed. At the same time, it was also collected samples from the raw domestic wastewater (after screen bars). All samples were collected on the same data at the same time and analyzed at each sampling point. The wastewater samplings were undertaken always in the morning: 8:00 to 10:00 hours using polyethylene bottles and kept refrigerated (4ºC) during transportation to the Water and Wastewater Analysis Laboratory at Polytechnic Institute of Castelo Branco, where analyzes were processed within 24 hours and always maintained at temperatures of 4 ºC.

Samples were analyzed for biochemical oxygen demand (BOD_5), chemical oxygen demand (COD) (COD_t and soluble, COD_s), total suspended solids (TSS), pH, total alkalinity (Talk), electrical conductivity (EC) at 20ºC, total nitrogen (TN), ammonium-nitrogen (NH^+_4-N), organic-nitrogen (Org-N), oxidized-nitrogen (NO^-_2-N; NO^-_3-N: NO_x-N), total phosphorous (TP) and total dissolved phosphorus (DP) in every wastewater samples. TN was calculated as the sum of the measurements for Org-N, NH^+_4-N and NO_x-N. For BOD_5 determination, WTW Manometric BOD Measuring Devices OxiTop® respirometric method was used. For all others wastewater quality parameters were performed according to the Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WEF, 2005). Soluble COD (CODs) was measured after filtration of the sample on filter membrane with mesh size of 0.45 µm. Samples were analyzed in duplicate.

For flow measurements, the WWTP had installed an ultrasonic flow meter, associated with V-notch weirs, just off the septic tank (which corresponds to the entrance of the macrophytes bed). The data relating to average daily flow rate of affluent to the wetland bed over the different months during the sampling period, were provided by the company *Águas do Centro, S.A.,* responsible of management and operation of the WWTP. The HLR (cmd^{-1}) was calculated dividing the acquired inflow rate ($m^3\ d^{-1}$) by the surface bed. The HRT (days) was calculated by dividing the pore water volume (m^3) by the inflow rate ($m^3\ d^{-1}$). Results are presented in Table 4.1.

Table 4.1 HLRs, HRTs and corresponding inflow rates for the CW system during the monitoring period

Months	**Average inflow rate ($m^3\ d^{-1}$)**	**HLR [a)] ($cm.d^{-1}$)**	**HRT [b)] (days)**
Aug.-12	98.9	23	2.6
Sep.-12	90.0	21	2.8
Oct.-12	135.4	32	1.9
Nov.-12	211.7	50	1.2
Dec.-12	116.8	27.4	2.2
Jan.-13	125.4	29.5	2.1
Feb.-13	115.9	27.2	2.2
Mar.-13	214.8	50.5	1.2
Apr.-13	91.9	21.6	2.8
May.-13	75.7	17.8	3.4
Jun.-13	65.3	15.3	4.0

a) For area using the porosity of the wetland media
b) For Pore water volume

The ESACB meteorological station, located at 15 km of the system study, was consulted for meteorological data of rainfalls and temperatures that were used to evaluate the influence of weather conditions on the treatment performance of the Sarnadas Rodão WWTP. To acquire information about the possible influence of seasonality on the performance of the system the data set of system was divided into two subsets: Spring-Summer (August 2012 and April to June 2013) fall-winter (September-December 2012 and January to March 2013), that correspond to warm and cold periods, respectively.

The RE (%) of pollutants were calculated as the ratio of the difference of the inflow and outflow concentrations by the inflow concentrations. Mass loading rate ($g.m^{-2}.d^{-1}$) was estimated by multiplying the inlet concentration ($g\ m^{-3}$) and the inflow rate ($m^3\ d^{-1}$) divided by the total wetland bed area (m^2).

4.2.3. Statistical analyses

All statistical analyses were performed using SPSS version 21 for windows. Prior to the analysis, the data were subjected to normal distribution and homogeneity of variance with a Kolmogorov-Smirnov with Lilliefors Significance Correction and with Shapiro-Wilk W tests ($n \leq 50$). The combined non normal distribution and a constrained sample size for the data led us to use non-parametric Mann-Whitney U tests to determine differences between inflow and outflow values for the different parameters analyzed in each step of treatment and for each monitoring period. Since the raw data were not normally distributed, non-parametric Kruskal-Wallis ANOVA tests were carried out to test the differences between both concentrations in the influent and effluent for both seasonal periods and also for analysis of the significance of differences between average efficiencies recorded for the periods of spring-summer and autumn-winter. The statistical analysis was conducted at a 95% confidence level.

4.3. Results and discussion

4.3.1. Climatic and hydraulic conditions

Figure 4.2 shows the average monthly rainfall and air temperatures occurring during the study period. Precipitation was marked by heavy rainfall in autumn, then decreased between December and February, but in March was recorded again a strong rainfall, with monthly value of 222.1 mm, which correspond to the highest monthly rainfall during the study period. The lowest monthly rainfall was recorded in June 2013 (9.2 mm). Precipitation was higher during cold period compared to warm period. Monthly precipitation during cold period ranged between 30.4 mm to 222.1 mm, whereas during spring-summer seasons it varied from 9.2 to 11.0 mm. Total precipitation during the dry season was 76.7 mm, while the rainy seasons had 853.7 mm. The monthly mean air temperature ranged between 8.1 to 24 ºC along the sampling period (August-12 and June-13). In the cold period (autumn-winter), monthly mean air temperature varied from 8.1 to 22.3 ºC, with an average of 12.1 ºC, while in warm period (spring-summer) the monthly mean air temperature was 19.8 ºC and ranged between 13.0 to 24 ºC.

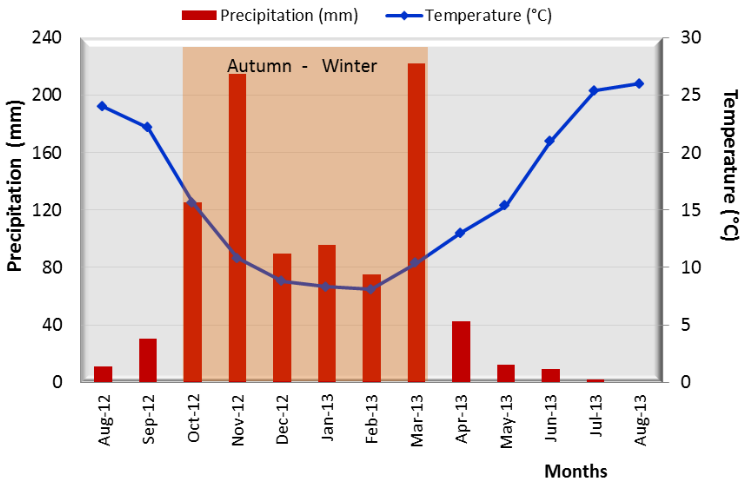


Figure 4.2 Total monthly precipitation and monthly averaged air temperature in Sarnadas Rodão (August 2012 to August 2013).

Figure 4.3 shows the variation of average daily flow rate and HLR over time. The values fluctuated from 15.3 cm d^{-1} (June 2013) to a maximum of 50.5 cm d^{-1} (March 2013), with an average of 28.7 cm d^{-1}, which caused a HRT between 1.2 and 4 d with a mean value of 2.4 d during the 11 months of the monitoring system, also lower than expected value (3.5 d) for the horizon-year. On average, the HLR applied to HSSF bed was about 1.7 times greater than the maximum value (17 cm d^{-1}) considered in the project for the horizon-year. Average HLR of 33.9 and 19.4 cm d^{-1} were observed during cold and warm periods, respectively, while for HRT, the values obtained for those periods were 1.2 and 3.2 days, respectively.

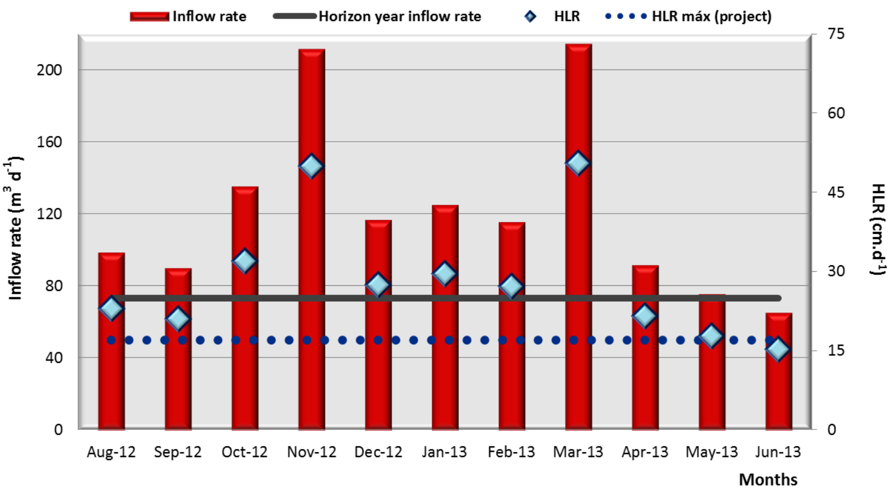


Figure 4.3 Variation of daily wastewater flow rate and hydraulic loading rates at HSSF bed of Sarnadas de Rodão along the monitoring period.

Those fluctuations on HLR can be explained not only by high stormwater flows observed especially during the autumn-winter period, but also by the area per inhabitant equivalent (2 m^2/p.e.) that was considered in the sizing of the system under study, which is lower than recommended by the International design criteria for HSSF-CWs systems (3 to 6 m^2/p.e.) (IWA, 2000; Rousseau *et al*., 2004; Kadlec & Wallace, 2009). According to Kadlec & Wallace (2009) and Vymazal (2010), these values are also outside the range of values considered appropriate (TRH: 5-15 days; HLR: 2-20 cm d^{-1}) to ensure an effective treatment by the HSSF-CWs.

Average inflow rate varied monthly, with values ranging between 65.3 and 214.8 $m^3 \cdot d^{-1}$. Observing Figures 4.2 and 4.3, it can be seen that the monthly variations in wastewater average inflow rate following the precipitation changes over time, which suggests that precipitation can significantly affect the total wastewater inflow entering in the system during the rainy season and hence will also influence the HLR.

It appears that the inflow rate registered at the entrance of the bed during the period of spring-summer, can be seen essentially as dry weather flow rate of domestic wastewater (on average 82.9 $m^3.day^{-1}$), which was around 22% greater than the maximum allowed in the project (73 m^3. day^{-1}). In the rainiest months, the average flow rate was 144.3 $m^3.day^{-1}$, which is about two times greater than the maximum value of the project. During the whole period, the inflow rate is above the horizon-year inflow rate (black curve), meaning that the system is undersized.

4.3.2. Treatment performance of Sarnadas Rodão HSSF-CW

The average physico-chemical characteristics for all parameters are shown in Table 4.2.

4.3.2.1. pH, total alkalinity and Electric Conductivity (EC)

The mean raw inflow pH was 7.1± 0.3 for entire sampling period (Aug-12-Jun-13), 7.0±0.2 and 7.2±0.4 for spring-summer and autumn-winter, respectively, and no significant seasonal differences were found along the three sampling periods considered. Akratos & Tsihrintzis (2007), in the longer term evaluation of HSSF wetlands operating with synthetic wastewater treatment did not also observed significant differences during the monitoring period between different seasons. Over the entire sampling period was observed significant differences ($p<0.05$) between the pH of raw inflow and pH at inlet and outlet of CW system, although no significant differences have been observed between the inlet and outlet of the wetland bed.

However, it was found that the pH tended to decrease throughout the treatment, with the average pH value of the effluent at the outlet of wetland bed dropped to 6.8±0.3. Similar trend was observed for the autumn-winter period. In fact, mean raw influent pH during cold period showed a significant ($p<0.05$) decreased after primary treatment (6.9±0.3), but the results also show that there are no significant differences ($p>0.05$) in pH value between the inlet and outlet sampling points from CW system. For spring-summer period, the mean value of pH has shown that there were no significant differences between the three sampling points, despite having also observed a slight decrease in pH values after passing through the HSSF system. The formation of organic acids associated with anaerobic degradation, the main form of degradation in HSSF-CWs systems, may explain this decrease as well as the occurrence of nitrification process within the bed that promotes the consumption of alkalinity.

Both, in the warmer seasons and in coldest the pH values have changed slightly along each one of those periods, as shown by lower values obtained for the standard deviation, although effluent pH in spring-summer period were higher (*$p>0.05$*) compared to autumn-winter season. The denitrification rate increases with temperature until the optimum value of 40 ° C (Metcalf & Eddy, 2003). As the ambient temperature was higher in the spring-summer period, the higher pH value in the effluent can be explained by a higher denitrification rate, which is the source of alkalinity, about 3 g of bicarbonate per gram NO_3^--N that is reduced to N_2 (Saeed & Sun, 2012). Sharma *et al*. (2013) also reported an increase in effluent pH during warm period when compared with cold period. The pH range of values are suitable for nitrification (6 to 9 are the required values) and for denitrification (7.0 to 7.5 are the required values) (USEPA, 2000).

EC is a numerical expression of its ability to carry an electric current. The mean values of EC observed at the inlet and the outlet of the bed was 448.7±282.9 $\mu S.cm^{-1}$ and 448.8±265.2 $\mu S.cm^{-1}$ in the entire monitoring period (August 2012-June 2013), 680.4±282.9 $\mu S.cm^{-1}$ and 652.0±251.5 $\mu S.cm^{-1}$ in spring-summer period and 283.2±129.3 $\mu S.cm^{-1}$ and 303.6±166.8 $\mu S.cm^{-1}$ in autumn-winter period. No statistical significant differences were found between the three sampling points for each monitoring periods. However, significantly ($p<0.05$) higher values was observed in the final effluent in the spring-summer period (about two times higher than in the same sampling point in autumn-winter), which may be explained by the increased in the mineralization rate of organic matter present either in the influent either accumulated inside the bed in winter-autumn period, as a result of the most favourable temperatures to the activity of microbial decomposers and also because of greater evapotranspiration rate due to the higher temperatures, which together can provide greater ion concentration in the final effluent in the warmer seasons.

Table 4.2 Characteristics (Mean ±; standard deviations) of the wastewater in the CW of Sarnadas Rodão for the three monitoring period and at each sampling point

Parameters	**Aug12-Jun13**			**Spring-Summer**			**Autumn-Winter**		
	Raw Inflow	Inlet	Outlet	Raw Inflow	Inlet	Outlet	Raw Inflow	Inlet	Outlet
pH	7.13±0.31^{a}	6.94±0.24^{b}	6.77±0.29^{b}	7.00±0.21^{a*}	6.96±0.22^{a*}	6.94±0.18^{a*}	7.23±0.35^{a*}	6.93±0.28^{b*}	6.64±0.30^{b*}
T_{alk}. (mg $CaCO_3$.L^{-1})	83.88±53.65^{a}	79.43±52.58^{a}	72.94±47.47^{a}	111.36±60.85^{a*}	116.00±58.27^{a*}	103.60±53.43^{a*}	64.26±41.59^{a**}	53.30±29.90^{a**}	51.04±29.77^{a**}
EC (µS.cm^{-1})	424.29±292.81^{a}	448.72±282.92^{a}	448.77±265.24^{a}	665.00±295.46^{a*}	680.40±282.87^{a*}	652.00±251.48^{a*}	252.36±127.36^{a**}	283.23±129.33^{a**}	303.60±166.76^{a**}
CODt (mg O_2.L^{-1})	286.96±68.89^{a}	205.39±47.03^{b}	106.08±17.12^{c}	286.10±95.05^{a*}	201.48±64.13^{b*}	98.94±25.06^{c*}	287.57±51.73^{a*}	208.19±35.93^{b*}	111.19±6.79^{c*}
CODs (mg O_2.L^{-1})	182.67±40.84^{a}	131.34±24.77^{b}	78.82±16.85^{c}	165.86±44.36^{a*}	135.86±34.36^{a*}	76.74±18.37^{b*}	194.67±36.64^{a*}	128.11±17.55^{b*}	80.30±17.01^{c*}
BOD_5 (mg O_2.L^{-1})	237.72±56.45^{a}	151.47±44.52^{b}	15.76±4.07^{c}	240.40±78.70^{a*}	157.28±55.52^{b*}	15.28±5.32^{c*}	235.80±41.26^{a*}	147.31±39.12^{b*}	16.10±3.35^{c*}
TSS (mg.L^{-1})	39.58±13.65^{a}	26.25±10.74^{b}	10.76±5.07^{c}	52.52±8.17^{a*}	36.14±6.43^{b*}	14.78±4.32^{c*}	30.34±7.61^{a**}	19.19±6.64^{b**}	7.89±3.42^{c**}
Org-N (mg.L^{-1})	3.19±0.75^{a}	1,86±0,54^{b}	1.31±0.42^{b}	3,72±0,78^{a*}	1.87±0.50^{b*}	1.40±0,45^{b*}	2.80±0.47^{a*}	1.85±0.61^{b*}	1.24±0.42^{b*}
NH_4^+-N (mg.L^{-1})	15.82±13.38^{a}	11.87±11.00^{a}	11.19±9.48^{a}	24.05±15.76^{a*}	21.02±11.36^{a*}	18.83±9.30^{a*}	9.94±8.10^{a**}	5.33±4.03^{a**}	5.74±4.87^{a**}
NO_x-N (mg.L^{-1})	1.58±0.6^{a}	1.38±0.44^{a}	1.44±0.53^{a}	1.64±0.38^{a*}	1.16±0.28^{a*}	1.12±0.39^{a*}	1.54±0.74^{a*}	1.53±0.49^{a*}	1.68±0.52^{a*}
TN (mg.L^{-1})	20.41±13.89^{a}	15.09±10.95^{a}	14.05±9.38^{a}	29.14±16.76^{a*}	23.97±11.64^{a*}	21.48±9.40^{a*}	14.18±7.59^{a**}	8.74±4.07^{b**}	8.74±4.87^{b**}
TP (mg.L^{-1})	2.46±2.75^{a}	1.79±1.42^{a}	1.38±1.06^{a}	4.62±3.10^{a*}	2.87±1.23^{a*}	2.08±0.82^{a*}	0.93±0.91^{a**}	1.01±1.00^{a**}	0.88±0.96^{a**}
DP (mg.L^{-1})	1.79±2.13^{a}	1.20±1.28^{a}	0.96±0.97^{a}	3.34±2.56^{a*}	2.26±1.34^{a*}	1.67±1.05^{a*}	0.68±0.73^{a**}	0.44±0.44^{a**}	0.44±0.50^{a**}

Note: Within each monitoring period, a different letter in superscript for a particular parameter indicates a statistically significant difference (p<0.05) between the sampling points; for a particular parameter, different number of asterisks indicate statistical significant differences between the two seasonal periods per sampling points.

4.3.2.2 Total Suspended Solids (TSS) and organic matter (BOD_5, CODt and CODs) removal

Influent TSS was reduced from 26.2 $mg.L^{-1}$ to 10.8 $mg.L^{-1}$ for the whole monitoring period and from 36.1 $mg.L^{-1}$ to 14.8 $mg.L^{-1}$ for spring-summer period, while for autumn-winter influent TSS concentration reduced from 19.2 $mg.L^{-1}$ to 7.9 $mg.L^{-1}$. The results showed that, for each monitoring period, significant differences ($p<0.05$) were observed in the TSS concentration values between the three sampling points throughout the treatment system. TSS increased throughout the spring-summer period, that would not be expected since the heavy rains that have been felt in the months of autumn-winter (Figures 4.2 and 4.3) should have contributed to an increase in the flow velocity of wastewater through the bed and hence one would expect that physical processes responsible for removing of TSS are adversely affected, resulting in higher values in TSS concentration than those found in the spring-summer period.

Figure 4.4 shows through the boxplot diagrams the TSS, BOD_5, COD_t and COD_s concentrations reduction between the inlet and outlet of wetland bed, for the three sampling periods. In each box-and-whisker plot the five horizontal lines proceeding from top to bottom represent the sample maximum value, upper quartile (75%), median, lower quartile (25%) and minimum value, and outliers are shown. The mean removal percentage of TSS after primary treatment were 33.7%, 31.2% and 36.8% for whole coverage time, spring-summer and autumn-winter periods, respectively, while the average removal percentage between inflow and outflow of HSSF studied was around 60% for all three monitoring periods. Thus, no ($p>0.05$) seasonal differences was observed in TSS removal efficiency, which is similar to the finding of other authors and attributed to the fact that in HSSF system TSS are mainly removed by sedimentation and filtration processes which are not significantly affect by temperature (Kadlec & Wallace, 2009; Llorens et al., 2009; Sharma et al., 2013).

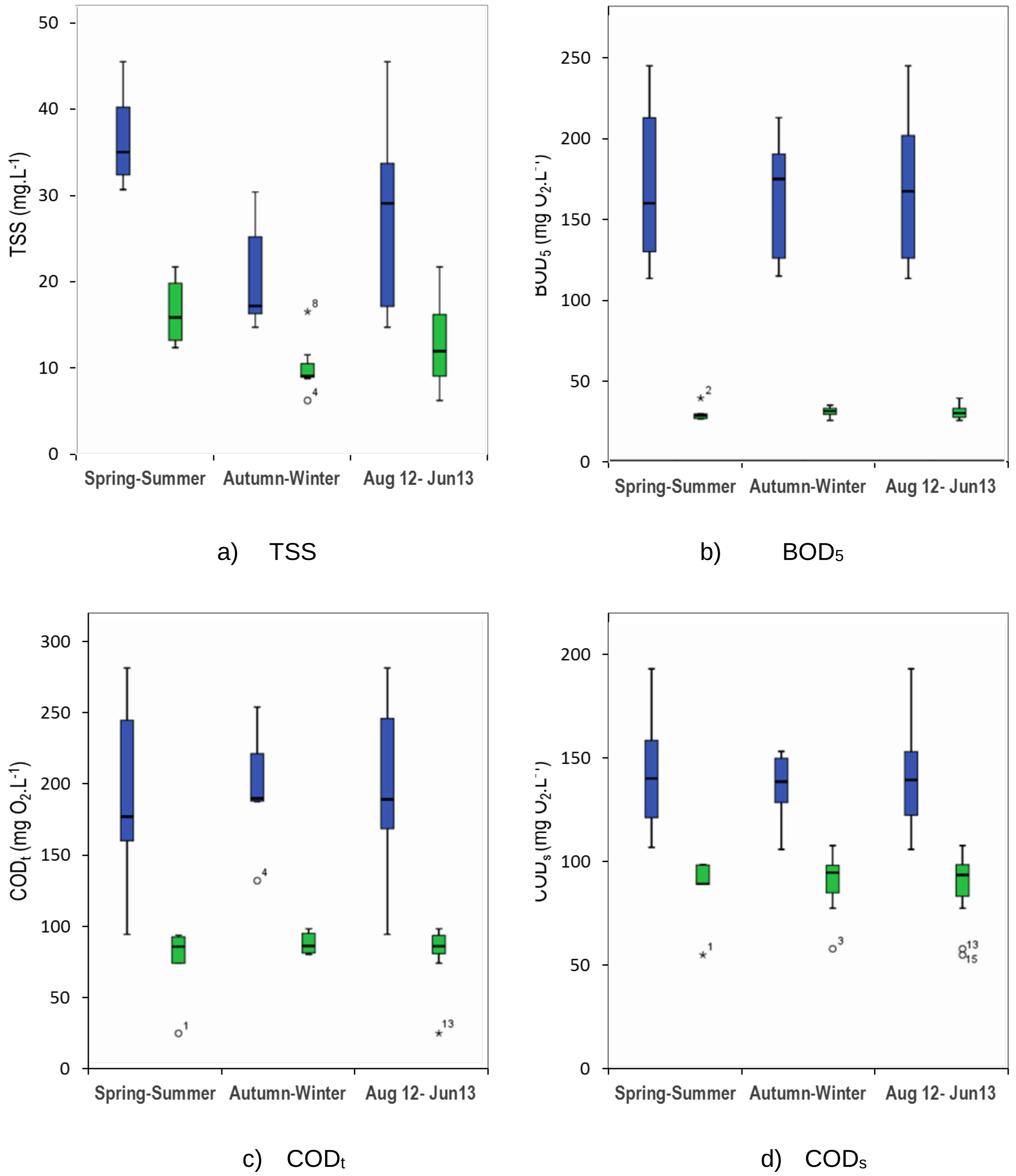


Figure 4.4 Boxplot diagrams of inflow and outflow concentrations of wetland system for whole monitoring period and for each season period.

Duarte *et al*. (2010) in a study involving 10 Portuguese HSSF systems of different climatic zones reporting efficiencies ranging between 55% and 97%, values which are similar to those (65%-97%) reported by Pascoal & Sousa (2013) who studied 25 HSSF systems distributed by the Central Region of Portugal, based on data provided by the operators responsible for the operation of the systems. Also Oliveira (2007) studied four CWs, located at Portugal's North Region and obtained as average values of TSS removal rates ranging from 60% -94%. In the Alentejo region, Galvão (2009) characterized the performance of two HSSF-CWs and obtained an average value of 75% for Fataca WWTP, having observed a slight seasonal difference in removing TSS (78%

for summer and 71% for winter), while for the other WWTP studied (Malavado) she has obtained the average value of 80% for the removal efficiency. Just like in the present study, also, Albuquerque *et al*. (2009) evaluating the operation of Capinha HSSF-CW, located in Central Interior region of Portugal and planted with *Pragmites australis*, observed that TSS removal was lower than expected (56.4%).

The average removal efficiency obtained for the system under study could be considered low since it is at the lower end of the range values usually reported in the literature (60%-90%) (Garcia *et al*., 2010; Vymazal, 2011), and one of the factors which may explain this low removal rate is the high HLR observed over the whole monitoring period, even during the warmer seasons (Table 4.1; Fig. 4.3), which were always higher than the maximum value considered in the project for the horizon-year (17 $cm.d^{-1}$) and are always outside the range considered appropriate to ensure an effective treatment by HSSF-CWs systems (IWA, 2000; Kadlec & Wallace, 2009; Vymazal, 2010).

Figure 4.5 presents the boxplot diagram of the overall removal efficiency of TSS over the different monitoring periods considered at this study and we can see that the system showed a lower seasonal variability. The figure also show that at autumn-winter the data are a more uniform distribution than at spring-summer that presented a more skewed distribution, indicating a greater variability in the TSS removal rate during this last seasonal period.

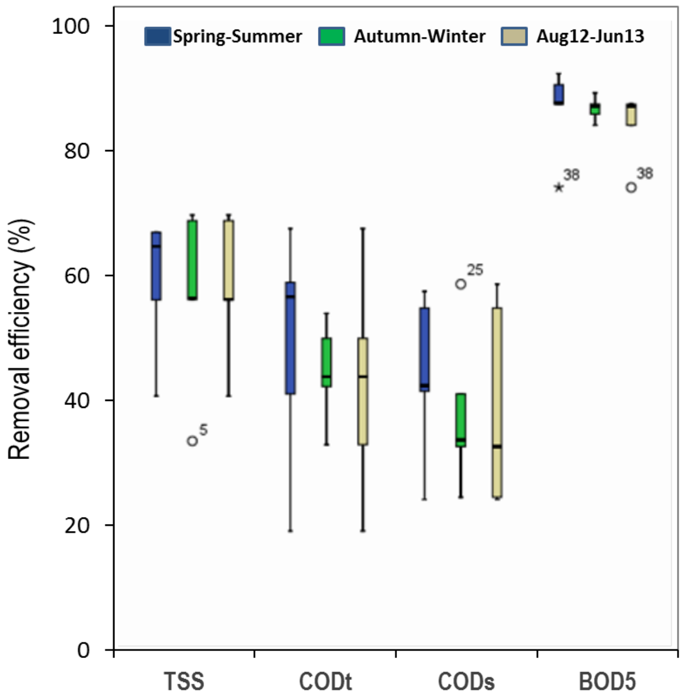


Figure 4.5 Removal efficiency of TSS, CODt, CODs and BOD_5 taken along the three different monitored periods.

Most of the solids tend to increase with increasing HLR (IWA, 2000; Ghosh & Gopal, 2010). In fact, plot of HLR against TSS removal shows that RE tend to decreases as HLR increases, despite TSS removal percentage showed a weak linear relationship with HLR for the three monitoring periods as shown by the very low values obtained for determination coefficient (R^2) (Aug12 - jun13: R^2=0.0024, *p-value*= 0.886; spring - summer-period: R^2=0.2609, *p-value*=0.379; autumn - winter: R^2=0.2818, *p-value*=0.279). Amado *et al*. (2012) in a study on the influence of stormwater infiltration on the treatment capacity of a LECA-based HSSF-CW in Interior region of Portugal showed that stormwater infiltration led to a high variation of the HLR throughout the bed, which has affected its performance in the removal of organic matter, suspended solids and nitrogen and for a HLR below 20 cm. d^{-1} they observed that the removal efficiencies for BOD_5, COD and TSS doubled.

Despite the HLR may have influenced the treatment efficiency, the low percentage of TSS removal in the present study may also be due to low total solid loading rate (SLR) in the affluent to the bed and whose values, on average, for the three sampling periods were 2.86 gTSS.m^{-2}. day^{-1} (Aug12 - Jun-13), 2.68 gTSS.m^{-2}.day^{-1} (spring - summer) and 2.47 gTSS.m^{-2}.day^{-1} (autumn - winter). The relationship between SLR and outflow TSS concentration during whole monitoring period and both seasonal periods considered in the present study also confirms this trend. No significant linear correlation (Aug12 - Jun13: R^2=0.1831, *p-value*=0.189) was observed between SLR and effluent TSS concentrations, although it has found that the TSS concentration in treated effluent showed a negative linear relationship with the SLR. Similar trend was observed during both season periods (spring - summer-period: R^2=0.1174, *p-value*= 0.572; autumn - winter: R^2=0.1012, *p-value*=0.539).

During the period under analysis, TSS was released consistently at levels below the recommended 60 mg.L^{-1}, maximum permissible limit for discharge to surface water courses in Portugal for WWTP with a population < 2000 inhabitants (Table 4.2; Figure.4.6), which was met for 100% of the collected samples, having the values in the treated effluent varied between 3.8 and 20.2 mg.L^{-1}.

The data show that for all monitoring periods it was observed a significantly ($p< 0.05$) decrease in the concentration of BOD_5, COD_t and COD_s between all sampling points (Table 4.2) for the three monitoring periods. However, the outflow concentrations of these different parameters are comparable ($p> 0.05$) for the two seasonal monitoring periods, despite the instability observed for the concentration of organic matter in the wastewater influent to the bed (Figure. 4.4), especially as regards to the BOD_5 concentration, in which it is observed values for the coefficients of variation (CV) exceeding 30%.

The statistical analysis on the results also showed CV in COD_t raw inflow of 24%, 33.2% and 18% for whole monitoring period and for warm and cold periods, respectively, which suggests that COD_t concentrations of the raw influent fluctuated over time, especially at spring-summer period (Figure 4.4). Similar trend was observed for COD_s. This might be related with seasonal fluctuations of the population, a characteristic feature of the villages of the rural area of Portugal, especially in the summer period when immigrants return for a holidays. Since during warm season there was no significant rainfall, this deviation may also be linked to the discharge into local sewer of effluents from small agro-livestock activities which are common in rural areas where it is located the WWTP under study.

Generally, high RE (> 88%) was recorded in the HSSF-CW for BOD_5 and for the three monitoring periods, values which are consistent with those reported in the literature for this type systems (Vera *et al.,* 2011; Vymazal & Kröpfelová, 2011; Garfi *et al.*, 2012; Hijoso-Valsero *et al.,* 2012), despite the mean value of BOD_5 loading rate applied to the system under study have been close to or above the recommended maximum value recommended by International design criteria (12 $gBOD_5.m^{-2}.day^{-1}$) depending on the sampling period (for whole monitoring period: 16.5 $gBOD_5.m^{-2}.day^{-1}$; warm period: 11.7 $gBOD_5.m^{-2}.day^{-1}$ and for cold period 18.9 $gBOD_5.m^{-2}.day^{-1}$). According to Chazarenc *et al*. (2007), it is recommended an OLR between 8 and 10 $gBOD_5.m^{-2}.day^{-1}$ for secondary treatment of domestic wastewater, values that are also lower compared with those of OLR average values recorded at the wetland bed.

The statistical analysis were carried out to determine the relationship between BOD effluent concentration and BOD loading rate and a significant ($p< 0.05$) positive linear central tendency regression was observed between these two variables with a coefficient of determination (R^2) of 0.864 and a *p-value* = 0.022 in spring-summer period. Similar trend was observed for the whole monitoring period and autumn-winter period although the coefficients of determination obtained in the adjustment of linear equations between the two variables under study for those periods (R^2=0.016, *p-value* =0.716; R^2=0.027, *p-value* =0.756, respectively) have been much lower than those obtained for the spring-summer period.

These results seem indicate that the residual concentration of BOD in the effluent of HSSF wetland systems could be influenced by the inlet concentration. In fact, in terms of influent organic loading it has been found that generally influences the wetland performance and normally RE tend to increase as organic loading increases and it is observed that tends correlate positively with mass loading rates when the systems were not overloaded in terms of influent organic loading (Ghermandi *et al*., 2007; Sharma *et al*., 2013).

Evaluation of the plots of HLR versus removal percentage of BOD_5 indicates that this removal tend to decrease with increased HLR, even though the effect of hydraulic loading on removal efficiency of BOD_5 has only been statistically ($p< 0.05$) significant for spring-summer period with a R^2 =0.794 and a *p-value*=0.042. This performance is consistent with the results reported by others authors (Rousseau *et al.*, 2004; Langergraber *et al*., 2007). The higher BOD_5 RE can be explained by the high biodegradability of organic matter present in the affluent to the wetland bed, which showed an average ratio of BOD_5/CODt around 0.7 for all periods of monitoring, indicating that organic matter are easily degradable by microorganisms (Metcalf & Eddy, 2003). At outlet, the BOD_5/COD_t ratio fell to values ranges between 0.1 and 0.2, as would expect in a domestic effluent after an effective secondary treatment (Metcalf & Eddy, 2003).

The existence of seasonal differences in RE is not consensual, although, generally, the results reported in the literature point to that a better removal is achieved in the period with the higher temperatures and during the growth cycle of the plants (spring-summer period for temperate regions) (Karathanasis *et al*., 2003; Solano *et al*., 2003; Arroyo *et al*., 2015). The higher growth and development of plants on the spring-summer period can also stimulated aerobic microbial activity by higher respiratory activity of roots.

In fact, Nivala *et al*. (2007) found higher organic removal in summer (60-90%), while during winter such removal ranges from 44% to 88% in an aerated HSSF-CW System and Fonder (2010) also observed a slight seasonal effect along two years, with higher removal rates on June and lower ones during the autumns and during the winters. Others authors also reported the negative impact of lower temperature on organic removal rates in the CWs (Akratos & Tsihrintzis, 2007; Olsson, 2011; Garfi *et al*., 2012). However, in these cases it is found that often the systems studied were lightly loaded and/or had long HRT, which can contribute for masking the effect of temperature. In our case, the wetland bed have a relatively short average HRT (2.4 days) and receiving a higher OLR over the entire study period, which may explain the fact that we did not find significant seasonal differences ($p > 0.05$) in the removal of BOD_5, although it was observed a BOD_5 organic load at the entrance of the bed 62.4% superior in the cold period when compared to the warm season.

Similar BOD_5 efficiencies to the ones found in this study were also shown by others Portuguese authors. Pascoal & Sousa (2013) in a study of 10 HSSF systems operating in the Central Region of Portugal, reported an average BOD_5 removal efficiency which ranged from 85.3% to 97.7%, while Duarte *et al.* (2010) pointed to RE of 70-95% when studied 20 full-scale HSSF-CWs in different climatic regions of Portugal. Oliveira (2007) conducted a study on four HSSF full-scale systems in the region of Northern Portugal during the spring-summer period and achieved removal rates ranging of the same order of magnitude (78-85%). Similar results were obtained by Galvão (2009) for two HSSF full-scale systems in Alentejo region of Portugal.

The Sarnadas HSSF displayed COD_t RE of 48.4%, 47.4% and 45.4% and 40.0%, 42.4% and 37.2% for COD_s considering the whole monitoring period, spring-summer and autumn-winter periods, respectively, which are lower than that found in other studies, generally >60% for CODt (García *et al.,* 2010; Vymazal & Kröpfelová, 2011). In comparison with average efficiencies reported by others Portuguese authors, we found that the average percentage of COD removal obtained for the system under study is usually lower than that reported for systems operated at full-scale in Portugal, which is generally greater than 70%. The differences observed can be mainly attributed to aspects of the criteria taken into account in the system design in the study, which will later reflect on different operating conditions in particular with regard area per population equivalent, HLR and HRT.

The lower average removal rate obtained in this bed can be associated to the average COD organic load that was relatively high for all sampling periods (Aug12 - Jun13: 22.4 $gCOD_t.m^{-2}.day^{-1}$; warm period: 14.9 $gCOD_t.m^{-2}.day^{-1}$; cold period: 26.8 $gCOD_t.m^{-2}.day^{-1}$) and generally outside the interval normally recommended both by International design criteria to size HSSF-CWs (5 to 20 $gCOD_t.m^{-2}.day^{-1}$) (IWA, 2000; Vymazal & Kröpfelová, 2008; Kadlec & Wallace, 2009) and as by German guidelines ATV-A 262 (16 $gCOD_t.m^{-2}.day^{-1}$) (Albuquerque *et al*., 2009).

No significant differences ($p > 0.05$) were observed between the three monitoring period; despite the tendency to the better removal result occur in the period with the higher temperatures and during the vegetative cycle of maximum development. Spring-summer period enhanced the COD_t and COD_s removal efficiency by 4.3% and 6.2%, respectively by comparing with autumn-winter period. Tuszyńska & Obarska-Pempkowiak (2008) observed a COD removal rate of 63.5% and 71.9% for BOD_5 during growing season, whereas in senescence period the removal rate decreased to 61.4% for COD and 70.3% for BOD_5. These results could potentially be related with the fact that most of COD_t in the affluent to the bed is in the form of particulate suspended solids which as aforementioned has no seasonal effect. In fact, approximately 63.9% of The HSSF influent COD_t was in particulate phase while 74.3% was observed in the effluent during whole monitoring period. Similar trend was observed for warm and cold periods where 67.4% and 61.5% of influent COD is in particulate form, while 77.6% and 72.2% of the effluent COD was in particulate form, respectively.

So, these results seems indicate that a significant amount of these recalcitrant organic compounds was not removed within the bed, even assuming that some of them come from the decay of plants (around 20%, according to Korkusuz, 2005). Taylor *et al*. (2011) observed differences in COD removal between planted and unplanted systems were more common and greater at cold temperature, showing that the role of plants may be more important from the point of view of improving RE at low temperatures. Sultana *et al*. (2015) in a study on the effect of temperature on COD removal observed that only in unplanted HSSF units the COD removal was significantly ($p < 0.05$) affected by temperature, having concluded that plants have the capacity to enhance COD removal along all the year via the oxygen release by roots and as its provide additional surface areas for microbial growth. They also verified that COD removal was limited after the cutting of plants, supporting the idea of the key role of plants in maintaining COD removal efficiencies in cold seasons.

The moderate performance of the bed regarding to COD removal was probably related to several reasons but could be also associated with several operative conditions, such as HLR and HRT. The entrance of high flow rate, especially during the autumn-winter where average flow rate was more 67% than the maximum value used to size the bed (73 $m^3.d^{-1}$) may negatively affect the performance of HSSF beds and the quality of the effluent, particularly with respect to COD removal because most of this being in particulate form as pointed above. These results are also in agreement with the bed behaviour regarding to the removal of TSS. During the 11 monitoring months the value of flow rate ranged from 65.3 m^3d^{-1} (Jue-13, driest month) to 214.8 m^3d^{-1} (March-

13, wettest month), which caused a variation of both HLR from 15.3 $cm{\cdot}d^{-1}$ to 50.5 $cm{\cdot}d^{-1}$ as aforementioned and of the HRT from 4.0 to 1.2 days. Therefore, the average HLR (28.7 $cm{\cdot}d^{-1}$) was around 69% higher than the respective project value for the horizon-year (17 $cm{\cdot}d^{-1}$) and average retention time in the CW was relatively short and is 2.4 days. These values are out the recommended values (2-20 $cm{\cdot}d^{-1}$ for HLR and 5-15 days for HRT) suggested in literature (IWA, 2000; Vymazal & Kröpfelová, 2008; Kadlec & Wallace, 2009). Thus, the HRT may not have been enough to ensure a sufficient contact time for the microorganisms to degrade organic matter present in the wastewater and moreover high values HLR may also have contributed to the entraining any particulate organic matter.

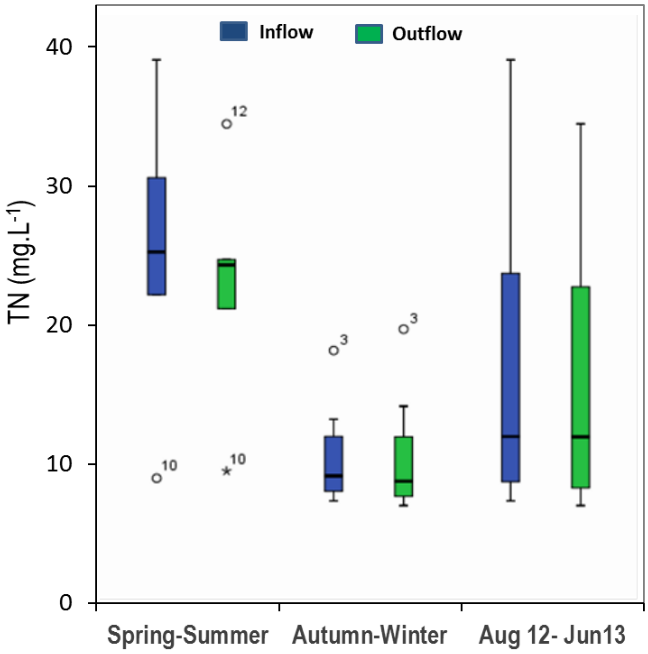
Such as for the BOD_5, the plots of HLR against COD_t and COD_s shows that removal efficiency of organic compounds tend to decrease with increased HLR, which is consistent to what the literature usually indicates (Rousseau *et al*., 2004; Chazarenc *et al*., 2007; Fonder, 2010), but no significant linear correlation was observed between the removal efficiency of COD_t and COD_s and HLR for all monitoring periods. Some authors reported that CWs can tolerate high variability in loading rates and sometimes an increase in organic loading rate seems to determine a higher organic removal (Calheiros *et al*., 2007; Sun & Saeed, 2009), although excessive loading rates can cause organic matter accumulation, reduction in void space and, consequently lower removal rates (Maltais-Landry *et al*., 2007).

Regarding the legal required discharge concentration values of BOD_5 and COD_t at the effluent, the CW system is operating according to the required by the licensing authority and performs an appropriate secondary treatment, according to Decree-Law No. 152/97 of 19th June. In both seasonal periods, for BOD_5, the maximum value measured at the outlet was 24.5 $mg.L^{-1}$, which respectful of the requested standard in Portugal (≤ 25 $mg.L^{-1}$) for discharging wastewater into natural bodies of water, while for COD_t the discharge standard is 125 $mg.L^{-1}$which also was achieved for 100% of the samples (Table 4.2 and Figure 4.4).

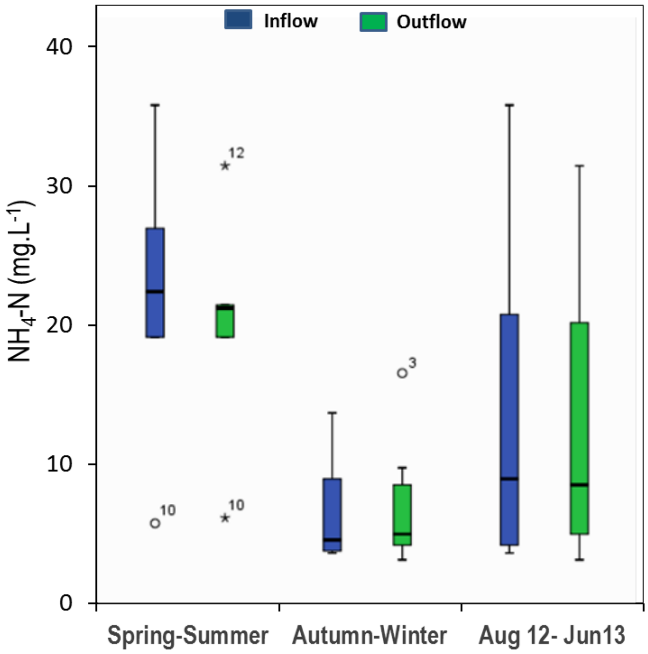
4.3.2.3 Nitrogen and phosphorus compounds removal

The concentration of the N compounds in raw inflow varied along the eleven months of monitoring, but on average the inlet concentrations are low. As might expected, NH_4^+-N is the N form that dominated in the affluent to the bed and accounted for average 79% of TN in whole monitoring period, 88% and 61% in warm and cold periods, respectively. The same trend was observed in the outflow of the CW bed with average NH_4^+-N concentrations accounted for 98.7% (Aug12 - Jun13), 87.7% (spring-summer period) and 65.7% (autumn - winter period) of TN.

Quite high coefficients of variation (CV) have been observed in raw inflow (68.1%, 23.5%, 84.5% and 37.9% for TN, Org-N, NH_4^+-N and NOx--N, respectively) which suggest that a significant change occurred in its characteristics over time. Also for the affluent to the wetland bed it was observed high fluctuation through time, especially for entire monitoring period and for spring-season period, as can be seen by the Figure. 4.6.



a)



b)

Figure 4.6 Variability of inflow and outflow TN (a) and NH_4^+-N (b) concentrations at Sarnadas Rodão system.

The quality of discharged wastewater was also unstable in time for all N compounds what was confirmed by very high CV with the values from 43.8% (warm period) 55.7% (cold period) and 64.7% (whole period) for TN and 49.4% (warm period), 84.8% (cold period) and 84.7% for NH_4^+-N (whole period). The same trend was observed for NO_x-N and Org-N, although the CV was lower and ranged from 31% to 37%. These results seem indicated that variability observed in the inlet concentrations was not significantly reduced in outlet, despite, in spring - summer period was observed lower variability in effluent quality when compared with autumn - winter period.

Compared with cold periods, average influent concentrations during warm period was higher ($p< 0.05$) for NH_4^+-N and TN, while average affluent of Org-N concentration was more and less similar for both periods and NO_x-N concentration was slight lower in warm period (Table 4.2). Effluent concentrations were also higher ($p< 0.05$) in warm period compared to colder seasons for NH_4^+-N and TN, but NO_x-N in contrast showed higher concentrations during cold period (Table 4.2). These results could be derived by dilution of the wetland inlet and outlet of the bed by rainfall during colder and rainy period and by the highest evapotranspiration rates in warm period that may also contribute to increasing the concentration of pollutants in the treated wastewater.

Over the whole time coverage (Aug12 - Jun13) the CW bed showed very low average removal efficiency for all N compounds. RE for TN, Org-N, NH_4^+-N and NO_x-N was 6.9%, 29.6%, 5.8% and -4.3%, respectively (Figure 4.7). These removals efficiencies were lower than those normally reported by the international and national literature (Rousseau *et al*., 2004; Mölle *et al*., 2005; Albuquerque *et al*., 2009; Kadlec & Wallace, 2009; Botequilha, 2013; Vymazal, 2010).

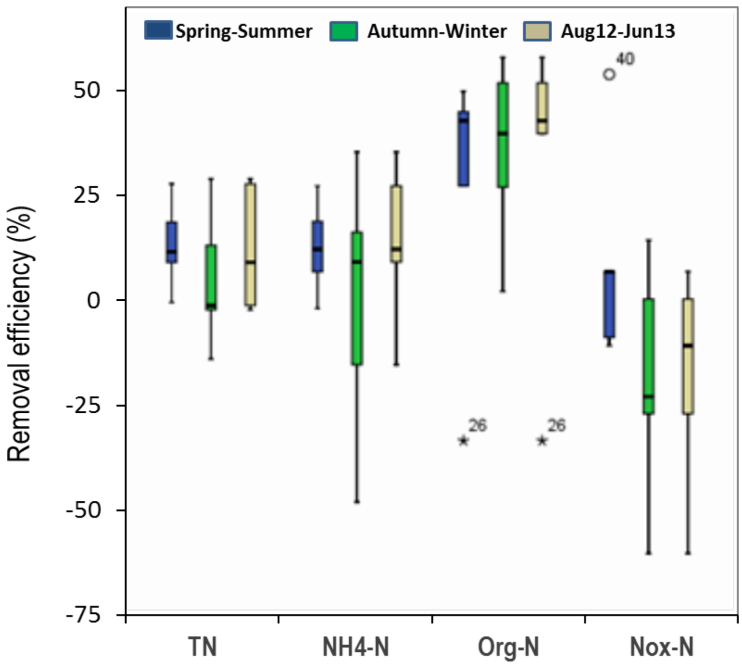


Figure 4.7 Removal efficiency for the different nitrogen compounds along the different monitoring periods.

Vera *et al*. (2011) in a study on the performance of 11 HSSF-CWs systems in Catalonia (Spain) reported a mean removal percentages for TN that are in the range of 48% and 66%, values that are consistent with the values found by Puigagut *et al*. (2007) who indicated a mean RE of 51% for TN in HSSF-CWs also in Spain. Mietto & Borin (2013) evaluated the performance of HSSF-CW treating domestic wastewater in northern of Italy in the first two years of operation and obtained a TN removal efficiency of 59%, for an HRT of 8 days.

Regarding the systems operating in Portugal, Duarte *et al*. (2010) point out RE of TN that ranging from 38.5% to 74.2%, when evaluated 8 full-scale CWs systems throughout of Portugal, while Simões (2009) reported a removal efficiency of 74.6%, 76.7% and 34.8% for TN, NH_4^+-N in a HSSF wetland system located in the same region of the studied system in this work. However, Oliveira (2007) in four HSSF systems operating in the northern region of Portugal, found an average removal efficiency of NH_4^+-N ranging between 4% and 51%, while Marecos do Monte & Albuquerque (2010) based in a nine-month campaign in an HSSF bed located at Interior Centre of Portugal, reported an average removal efficiency of 76.3% and 78.8% for TN and NH_4^+-N.

Figure 4.7 allows observing that the RE was more unstable in autumn-winter when compared to spring-summer period and although the values obtained are very low for both seasonal periods, we found that generally the average removal efficiency was higher for all parameters in the warmest period, except for organic nitrogen. A statistical analysis of the seasonality variable has shown there was significant effect of season in TN, NH_4^+-N and NO_x-N removal efficiencies. In fact, we observed that autumn-winter average removal efficiency were significantly ($p<0.05$) lower for TN (0%), NH_4^+-N (-7.7%) and NOx-N (-9.8%) than for spring-summer seasons for which the percentage of removal were, respectively, 10.4%, 10.4% and 3.4%.

These results are matching with some other studies which also found that nitrogen removal efficiencies in warm seasons tend to be higher than those obtained for colder periods (Hijoso-Valsero *et al*., 2012; Botequilha, 2013). These removal efficiencies were lower than that observed in similar system and climate conditions by Albuquerque *et al*. (2009) and by Marecos do Monte & Albuquerque (2010) also in Interior Central Region of Portugal, having the first authors reported a removal efficiency for TN, NH_4^+-N and NO_3^--N of 86%, 89% and 80%, respectively during a spring-summer period (May to August), while the second found an average removal efficiencies of 76% and 78.8% for TN and NH_4^+-N, respectively over a period from March to December.

Vegetation seems to play an important role in the effectiveness of wastewater treatment of HSSF-CWs and also as a factor in improving N removal performances, in spite of this improvement may vary with the different macrophytes species (Picard *et al*., 2005). The positive effects of plants in N removal is normally attributed to their role in root system uptake several inorganic compounds (e.g. N and P, that are categorized as macronutrients and that are contaminants presenting in wastewater to be treated) and, as aforementioned, also in release oxygen and organics compounds into the rhizosphere which seems to be crucial for stimulate the growth of some microorganisms involved in removal mechanisms of nitrogen compounds (Zhang *et al*., 2009; Taylor *et al*., 2011; Van de Moortel *et al*., 2010; Bialowiec *et al*., 2011; Kaseva, 2014).

Thus, highest removal percentage ($p < 0.05$) in removing NH_4^+-N and NO_x-N during the spring-summer period might be associated with greater absorption of these compounds by plants during this period due to coincide with the growing season and greater density of plants on the system under study, which in turn stimulates ammonia removal by the development of biofilms in plant roots. In fact, the system was vastly covered by vegetation that likely stimulation nitrogen uptake during spring-summer period.

The results clearly show a seasonal pattern in relation to the removal of TN, NH_4^+-N and NO_x-N with high removal at warm periods which highlighting the influence of temperature on the nitrification and denitrification processes. Similar behaviour were obtained by other authors (Akratos & Tsihrintzis, 2007; Burchell *et al*., 2007; Garfi *et al.*, 2012; Hijoso-Valsero *et al*., 2012; Silveira *et al*., 2015). Akratos & Tsihrintzis (2007) have reported that for wastewater temperature below 15 ºC the mean TN removal decreased, while Burchell *et al*. (2007) highlight the fact that with average temperatures of 7.5 ºC denitrification activity did not respond to added organic matter. The temperature between 20-25 ºC it has been referenced in several studies as being the optimum range for the denitrification process (Jing & Lin, 2004; Shao *et al*., 2013; Zhang *et al*., 2015). The mean air temperature in this study was of 12.1 ºC for cold period, which may indicate that the conditions of nitrification/denitrification were worse than those that occurred in the warmer months, for which was obtained a monthly mean air temperature of 19.8 ºC that could have benefited both nitrifying and denitrifying bacteria.

Generally, a HRT of 2 to 10 days have been reported to improve N removal in HSSF systems (Tuncsiper, 2007). However, Akratos & Tsihrintzis (2007) stated that at low temperature, an HRT of 8 days is not appropriate for nitrogen removal. In our case, the average HRT was 2.4 days which seemed insufficient taking into account the overall unsatisfactory removal efficiency for all nitrogen compounds analyzed and for the two seasonal periods. The negative average removal in NH_4^+-N in autumn-winter may also be related to high flushing due the high flow rate of water through the wetland bed during the rainy months.

As we have aforementioned, higher measured HLR were observed along the whole time coverage in the present study and we observed that lowest and negative removal efficiency for TN and NH_4^+-N was obtained for the months (October to March) in which it was observed higher HLR probably because the contact time between wastewater and biofilm responsible by the nitrogen removal is inadequate. Pan *et al.* (2012) also observed that when increased HLR the removal efficiencies of NH_4^+-N and TN decreased, which is also similar with what was found by others authors (Dan *et al*., 2011; Cui *et al*., 2015). Although lower HLR generally resulted in better removal efficiencies of N compounds, RE did not significantly ($p > 0.05$) correlate with HLR and the linear relationships between HLR as independent variable and removal percentage were rather weak for all nitrogen compounds and for all monitoring periods, with coefficient of determination (R^2) always less than 0.4356.

The lower removal of TN and NH_4^+-N might be also explained by the relatively small area of the bed considered in design project (~2 m^2/p.e.) and which also contributed to lower residence time. Wallace & Knight (2006) reported that to obtain significant reductions in N compounds the unit area values must be above 12 m^2/p.e., while Vymazal (2005) established that the value of 5 m^2/p.e. is recommended for eliminate organic matter and suspended solids, but it is insufficient to achieve complete nitrification. However, Vera *et al*. (2011) in a study on 11 HSSF-CWs in Spain did not found a clear and direct relation between TN removal and the surface area.

Several authors reported that increased in N loads within certain limits tend to contribute to grater removal rates in HSSF-CWs (Lee & Scholz, 2007; Paredes *et al*., 2007; Tuncsiper, 2007; Albuquerque *et al*., 2009; Marecos do Monte & Albuquerque, 2010; Dan *et al*., 2011). The results obtained have been shown that the concentrations of TN and NH_4^+-N in the effluent tend to significantly ($p < 0.05$) decreased with increasing respective incoming mass load rates, although the linear relationship between the two variables is moderate as shown by the values found for the coefficient of determination (TN: R^2 = 0.3738; NH_4^+-N: R^2 = 0.3521).

The same trend was observed for spring-summer period, despite not having found a significant linear relationship between the two variables neither for TN nor to the NH_4^+-N. For autumn-winter this tendency was less clear since the measured data were spread more widely in this seasonal period. Similar tendency were also observed for NO_x-N, despite the linear function used to describe the relationship between NO_x-N loading rate and respective effluent concentration it was not statistically significant ($p > 0.05$). For organic nitrogen in any trend curve could be drawn out of the results and thereby its concentration into the effluent does not seem to be affected by the inlet Org-N loading rate.

The quality of treated effluent was unstable in time and considering the discharge requirements imposed by Portuguese Decrees-law No. 152/97 and 236/98, during the spring-summer period, the average emission of N compounds (TN and NH_4^+-N) did not meet the legal limits. TN concentrations on the discharge site were 1.4 times higher than TN limit (15 mg L-1) and NH_4^+-N concentrations were 1.8 times higher than NH_4^+-N limit (10 mg.L^{-1}).

TP inlet concentration did not exceed 4.55 mg.L^{-1} along the entire monitoring period, although the concentrations values have changed throughout the study period (Figure 4.8) from 0.13 mg.L^{-1} (February and March) to 4.55 mg.L^{-1} (June). On average, during spring-summer and autumn-winter periods the TP concentrations in the affluent to the wetland bed were of 2.87±1.23 mg.L^{-1} and 1.01±1.00 mg.L^{-1}, respectively (Table 4.2). TP effluent concentrations from wetland bed were generally slightly lower than at the inlet, except on October, February and March. On average, HSSF bed does not provide statistically significant abatement ($p>0.05$) in TP concentration on any sampling periods considered, and effluent also maintaining a wide variability (Figure 4.8; Table 4.2).

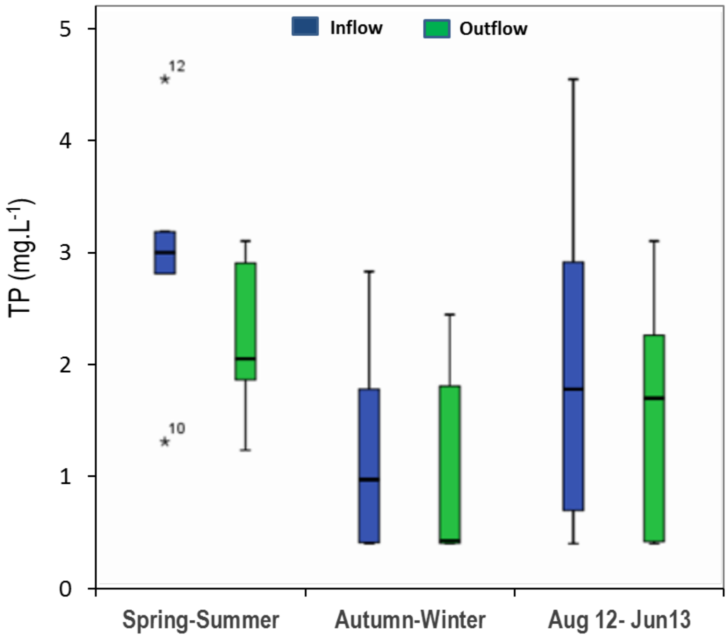


Figure 4.8 Seasonal variations of total phosphorus (TP) concentrations in inflow and outflow of the Sarnadas Rodão HSSF system during the study period and for the three monitoring periods.

HSSF bed system influent and effluent concentrations of dissolved phosphorus (DP) showed a pattern in accordance with TP concentration, however lower concentrations were measured at different sampling points (Figure 4.9; Table 4.2). No statistically significant difference ($p > 0.05$) was found between average influent and effluent concentration at the bed for the three monitoring periods (Table 4.2), despite the mean outflow DP concentrations were lower than the inflow concentrations.

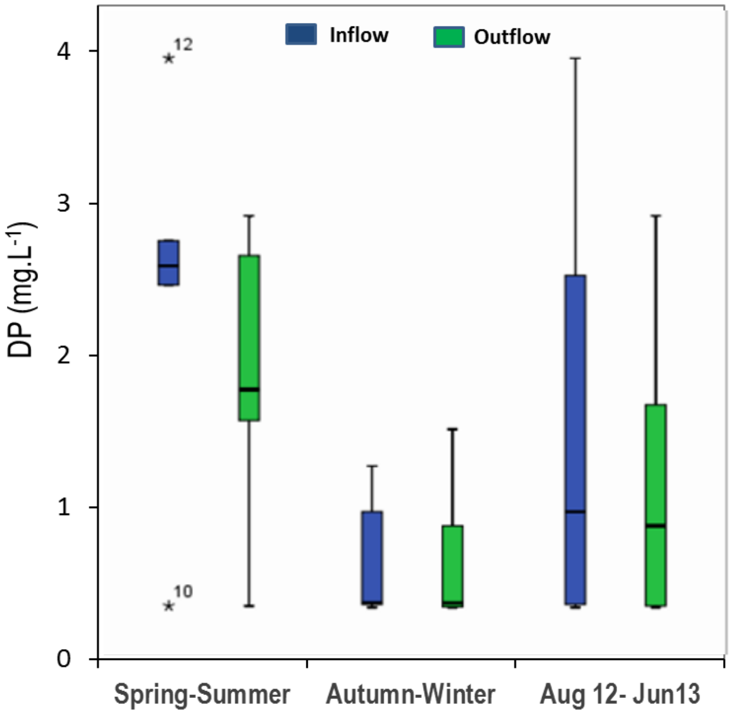


Figure 4.9 Seasonal variations of dissolved phosphorus (DP) concentrations in inflow and outflow of the Sarnadas Rodão HSSF system, during the study period and for the three monitoring periods.

Significantly lower mean values for the concentration of TP and DP were observed in autumn-winter period, either at the entrance or at the output of the bed, which may be explained by the dilution effect caused by rainfall during that period, while most evapotranspiration rate may account for the higher values found in the spring-summer period. In fact, in spring-summer period average concentrations of TP and DP in influent were respectively 2 and 5 times higher compared to autumn-winter period. Average effluent concentrations showed similar trend to influent, with highest values during spring-summer (2.5 and 3.5 times higher respectively to TP and DP). The average concentrations of TP in the effluent were below the discharge limit (10 $mg.L^{-1}$) according to the Portuguese Decree-law No. 236/98.

The RE of phosphorus compounds was relatively poor when compared with TSS and organic compounds, but better than N compounds removal. Average removal rates of TP were 23%, 27.5% and 12.9% in whole monitoring period, in spring-summer and autumn-winter periods, respectively. Regarding to the DP the system removed 20% along the entire sampling period, 26.1% in spring-summer period, and finally 0% in autumn-winter period.

Thus, the results allow us to conclude that there were significant differences ($p<0.05$) in the rate of removal of TP and DP between two seasonal periods considered, with highest percentage removal occurring in warmest period. The results showed that there was a sharp decrease of 14.6% in the removal efficiency of TP from spring-summer to autumn-winter, whereas regarding the DP; this reduction was of 26.1% (Figure 4.10). This figure also shows that there was a trend toward greater instability in the efficiency of removal of both the TP and DP during the autumn-winter.

The variety of P RE for domestic wastewater treatment observed in HSSF-CWs in Portugal is high and generally ranged from 26% to 94% (Oliveira, 2007; Simões, 2009; Duarte *et al*., 2010; Marecos do Monte & Albuquerque, 2010; Botequilha, 2013). These differences may be linked to differences in phosphorus concentration in the influent to the beds or on the difference in the HLR, HRT, material used for bed construction and how long the system is operated.

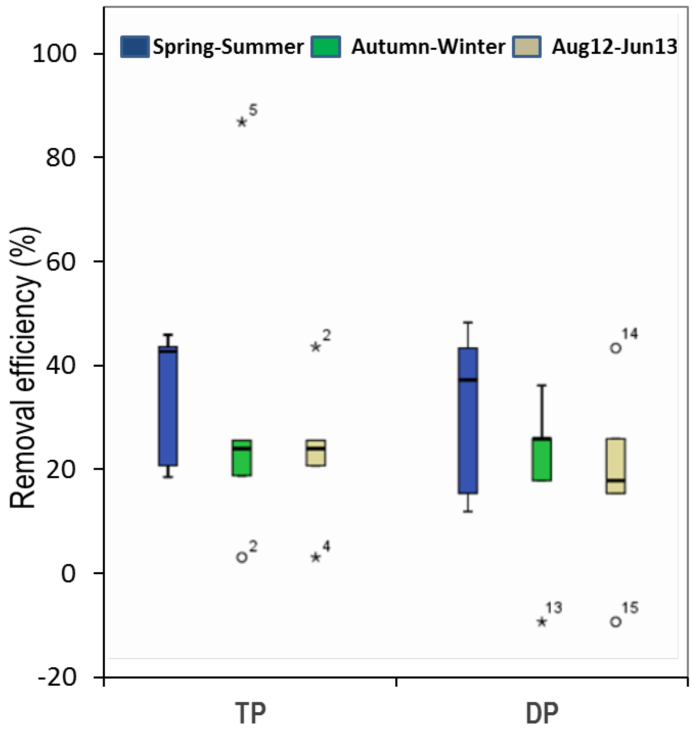


Figure 4.10 Removal efficiency for TP and DP along the different sampling periods in HSSF-CW at Sarnadas Rodão, Portugal.

The low TP removal efficiency observed in this study are not different from what is reported in literature (Garcia *et al*., 2010; Gagnon *et al*., 2012). Vymazal (2005) in a study based on data collected worldwide regarding the removal of P in HSSF-CWs reported an average mass-based efficiency of 32%, while Vymazal & Kröpfelová (2008) reported a better performance (50%) for systems operating in Europe. Vera *et al*. (2011) for 11 HHSSF-CWS in Catalonia, Spain was achieved a maximum mean of 58% for TP removal efficiency. Also Tanner & Sukias (2011) reported a poor P removal in three wetlands where DP was the dominant P form in the inflow and found in fact the wetlands acted as net sources of P on an annual basis during establishment. Generally, P RE reported in the literature is variable and usually range from 26 to 70% (Rousseau *et al*., 2004; Sharma *et al*., 2013; Zhang *et al*., 2015).

Stein & Hook (2005) consider that the seasonal performance patterns observed for TP and DP in several works could be explained by the potential seasonal variation in rhizosphere oxidation, which seems to promote the adsorption and precipitation of P. According Vymazal (2007) in HSSF-CWs there is little fluctuation in redox potential in the bed due to its substrate is constantly waterlogged, but generally it is assumed that oxygen transfer would be greater during periods of active plant growth (Hijoso-Valsero *et al*., 2012; Imfeld *et al*., 2009; Zhang *et al*., 2009).

Another possible explanation for higher removal efficiency in spring-summer period regarding autumn-winter can be linked to the fact that the average concentration of P (TP and soluble phosphorus) have decreased sharply in the winter time at the entrance of the bed, which could have contributed to an apparent release of accumulated P from the adsorption sites due to exchange ions reactions which might resulted into a sharp decrease in phosphorus removal efficiency. Similar results are also reported by other authors (Dong *et al.,* 2011; Sharma *et al*., 2013; Rai *et al*., 2015; Zhang *et al*., 2015).

P removal also occurred by via biological through assimilation by the microbial biomass and also by plants uptake. Xu & Shen (2011) reported that the biofilm growing within the substrate medium could be responsible by up to 31%-71% of the P removal from wastewater. Wu *et al*. (2014) in a study with four species of plants found that plants uptake varied between 10.8%–34.2% of phosphorus removal, while Cui *et al*. (2015) achieved that removal efficiency through plant uptake accounted only 0.43% of TP removal.

Similar to concentrations, high fluctuation in average inlet loads were observed for TP and for DP throughout the all study period. Average loads in influent to the CW bed was of 0.19 $g.m^{-2}.day^{-1}$ for TP and 0.13 $g.m^{-2}.day^{-1}$ for DP for the whole time coverage, whereas in spring-summer period were recorded a mean values of TP and DP loads applied to the system of 0.21 $g.m^{-2}.day^{-1}$ and 0.17 $g.m^{-2}.day^{-1}$, respectively which were higher than those observed for the autumn-winter period (0.13 $g.m^{-2}.day^{-1}$ for TP and 0.06 $g.m^{-2}.day^{-1}$ for DP). TP loading rate in this wetland bed was more than 6 times larger than the loading rate of 0.03 $g.m^{-2}.day^{-1}$ suggested by Crites (1994) for HSSF-CWs, but smaller than that considered at the project (0.44 $g.TP.m^{-2}.day^{-1}$).

The correlation between the TP concentration in effluent and inlet TP loading rates for entire sampling period has shown that there was a negative linear ($p > 0.05$) relationship ($R^2 = 0.315$, *p-value*=0.07), but for the two seasonal periods no trend curve could be drawn based on the results, probably due to the relatively few samples per period. Regarding to the DP, the same statistical analysis also showed a negative ($p > 0.05$) linear relationship between the loads of DP applied and the concentration of dissolved phosphorus in the treated effluent with $R^2=0.186$ and *p-value*=0.185 for entire coverage time, $R^2 = 0.381$ and *p-value*=0.268 for spring-summer period and $R^2=0.1134$ and *p-value*=0.514 for autumn-winter months. Also Maltais-Landry *et al*. (2007) in a mesocosm HSSF-CWs treating trout farm wastewater observed that TP removal efficiency decreased with increasing loading rates.

4.4. Conclusions

The results show that there was a significant reduction in most of the analysed pollutants entered into the Sarnadas Rodão HSSF-CW and the season period did not have a significant impact in most of the pollutant removal efficiencies. However, the removal efficiencies were different according to the parameters and to the seasonal periods. The removal of TSS and COD was lower than the ones reported in literature, which can be related to the low surface area per inhabitant considered in the design of the bed, as well as due to the fluctuations and high values of HLR. Nevertheless, the effluent concentrations of all pollutants fulfilled the national standards setup for the discharge of treated wastewaters into surface waters. The removal of nitrogen compounds was low, especially for TN and NH_4^+-N, and may be related to fluctuations in the HLR, which have affected the HRT, due to the entrance of stormwater into the local sewage system. Generally, average removal efficiencies for all nitrogen species were higher ($p<0.05$) in the warmest period when compared to those in the autumn-winter period, except for organic nitrogen. P removal (TP and DP) was higher ($p<0.05$) in spring-summer period, which may be related to plant uptake.

The natural systems that represent the beds macrophytes are characterized by their stochastic nature, which may explain the variability of the results that were observed over time. This study was performed during a limited (11 months) period and so, it is crucial to continue the study at full- scale of this type of CWs systems in order to assess and confirming temporal changes and performance evolution in such systems in Portugal.

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5. Effect of vegetation on the performance of horizontal subsurface flow constructed wetlands with lightweight expanded clay aggregates[4]

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Abstract

This research evaluates the effect of both organic and ammonia loading rates and the presence of plants on the removal of chemical oxygen demand and ammonia nitrogen in horizontal subsurface flow constructed wetlands, two years after the start-up. Two sets of experiments were carried out in two mesocosms at different organic and ammonia loading rates (the loads were doubled); one without plants (control bed), the other colonized with *Phragmites australis*. Regardless of the organic loading rate, the organic mass removal rate was improved in the presence of plants (93.4% higher for the lower loading rate, and 56% higher for the higher loading rate). Similar results were observed for the ammonia mass removal rate (117% higher for the lower loading rate, and 61.3% higher for the higher loading rate). A significant linear relationship was observed between the organic loading rate and the respective removal rates in both beds for loads between 10 and 13 g $m^{-2}.d^{-1}$. The presence of plants markedly increase removal of organic matter and ammonia, as a result of the role of roots and rhizomes in providing oxygen for aerobic removal pathways, a higher surface area for the adhesion and development of biofilm and nitrogen uptake by roots.

Keywords: Constructed wetland; lightweight expanded clay aggregates; loading rate; nitrogen removal; organic matter removal; *Phragmites australis*; subsurface flow

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5.1. Introduction

Constructed wetland (CW) systems with reeds and horizontal subsurface flow (HSSF) have been used for the treatment of domestic wastewater, industrial effluents, landfill leachate, polluted river water and stormwater runoff, among others (Juang & Chen 2007; Vymazal & Kröpfelová 2008; Vymazal 2009; Albuquerque *et al.* 2009a; Randerson *et al*. 2010, Bialowiec et al. 2012a). The main advantages of these systems include low costs of construction, maintenance and operation in comparison with activated sludge, anaerobic digestion or percolating filters for the same population size and similar flow rates and pollutant loads (Vymazal & Kröpfelová 2008; Kadlec & Wallace 2008). Therefore, nowadays there has been an increase in their use for domestic and wastewater treatment, as well as for advanced and polishing treatment if water reuse is an option (Masi & Martinuzzi 2007; Marecos do Monte & Albuquerque 2010; Pedrero *et al*. 2011a, b; Amado *et al*. 2012; Bialowiec *et al.* 2012a, b).

Although plants are one of the most important components of the wetland ecosystem, pollutant removal is also accomplished through an integrated combination of biological, physical and chemical processes. The substrate and the coupled microbial communities can remove a broad range of undesired constituents (organics, nutrients, heavy metals and solids) commonly found in wastewaters (Vymazal & Kröpfelová 2008; Lu & Huang 2010), through mechanisms such as filtration, sedimentation, biochemical pathways (*e.g.* aerobic respiration, nitrification, denitrification, anaerobic respiration and other non-conventional pathways), adsorption, precipitation, volatilisation and plant uptake (Vilpas *et al*, 2005; Paredes *et al*. 2007; Vymazal 2007; Kadlec & Wallace 2009; Vymazal 2009; Cheng *et al.*, 2011).

HSSF beds are the most widely applied CW systems due to the simple technology used, reliable operating conditions and good potential to remove moderate loads of organics, nitrogen, phosphorous and solids. Removal efficiencies above 90% are normally achieved for suspended solids and organic matter (such as chemical oxygen demand (COD) and up to 50% may be expected for nitrogen (Vymazal 2007, 2009; Kadlec & Wallace 2008). Light–expanded clay aggregates (LECA) can be used to improve the treatment capacity since they present both higher porosity and specific surface area, which allows a better biofilm adhesion and require smaller bed areas than the conventional gravel substrate (Albuquerque *et al*. 2009b; Bialowiec *et al.* 2011, 2012b).

The removal rates and performance of HSSF may vary over time and space and are dependent on multiple factors such as influent wastewater characteristics, hydraulic loading rate (HLR), organic loading rate (OLR), nitrogen loading rate (NLR), hydraulic residence time (HRT), bed maturity, media size, bed depth, plant species, among others (Stottmeister *et al*. 2003; Kadlec & Wallace 2008; Albuquerque *et al*. 2009a; Vymazal 2009; Bialowiec *et al*. 2012a, b). As with many other natural wastewater treatment systems, pollutant removal processes in a HSSF bed are affected by the variations of OLR and NLR that may produce a quick change in the removal rates of COD (r_{COD}) and ammonia nitrogen (r_{NH4N}) and this effect is not well studied for LECA-based beds. Such changes are also dependent on the oxygen transferred to the subsurface environment through roots or direct nitrogen assimilation by plants (Vymazal & Kröpfelová 2008; Bialowiec *et al.* 2012a). Vegetation presents a thermoregulatory effect (Kadlec & Wallace 2008; Brisson & Chazarenc, 2009; Bialowiec *et al*. 2012b) and this mechanism positively affects most biological pathways. On the other hand, the growth and development of roots and rhizomes provide surface for microbial growth, which benefits most of the microbiological removal pathways.

OLR, NLR, HLR and HRT are important variables for the design and operation of HSSF. The removal efficiencies of organics, nitrogen and phosphorous depend on the oxidation-reduction conditions, which are influenced by several factors including the applied loads (Kadlec & Wallace 2008; Albuquerque *et al* 2009b; Lu & Huang 2010). If the pollutant loading rate exceeds the oxygen transfer rate, the aerobic decomposition of organic matter and the nitrification process may be inhibited. Therefore, pollutant removal may be optimized by balancing the pollutant loading rate and the oxygen transfer. In general, low organic loads promote more oxidised conditions and therefore a better performance than high loads. The recommended loading rates range widely (Kadlec & Wallace 2008; Vymazal & Kröpfelová 2008), indicating the inherent dynamics and variability in natural treatment systems, but also the need for a better understanding of removal processes and improved design guidelines. Paredes *et al.* (2007) observed that NLR correlated strongly with effluent loading for ammonia nitrogen (NH_4-N), however, they also observed that NLR did not correlate with r_{NH4N}. Therefore, the influence of NLR on final effluent nitrogen concentrations and removal rates is yet unclear and not well known for LECA-based beds.

The inconsistency of the results found in studies with HSSF for the removal of COD and NH_4-N, as well as the effect of vegetation for different loading conditions (Jing *et al*. 2002; Akratos & Tsihrintzis 2007; Albuquerque *et al*. 2009a; Brisson, & Chazarenc 2009; Pedrero *et al.* 2011a; Amado *et al.* 2012) and the absence of studies for LECA-based HSSF-CW suggested the development of this research work. Therefore, the aim of this work is to evaluate the impact of reeds (*Phragmites australis*), OLR and NLR on the removal of COD and NH_4-N in LECA-based beds under the same HLR, and was developed from March 2009 to October 2010 at the Department of Civil Engineering and Architecture of the University of Beira Interior (Covilhã, Portugal).

5.2. Materials and methods

5.2.1. Experimental setup

Two HSSF with approximately 2 × 0.8 × 0.7 m (length × width × height) were run in parallel, one unplanted (control bed), the other planted with reeds (*Phragmites australis*). The effective surface areas were approximately 0.65 m^2 and both beds were filled with LECA aggregates (Filtralite NR aggregates with an effective diameter ranging from 4 to 8 mm, specific surface area of 1250 m^2m^{-3} and void ratio of 0.45). The planted bed was colonised two years before the experiments (*i.e.*, the plants were well developed during the experiments). The water table was fixed at 0.2 m. Three sampling points (PI2, PI5 and PI8) were used inside each bed to collect water samples for analytical measurements as shown in Figure 5.1, with the following lengths: PI2 (0.33 m away from the inlet), PI5 (1 m away from the inlet) and PI8 (1.9 m away from the inlet). The inlet device was composed by a perforated “T” tube connected to a peristaltic pump (Ismatec MCP-CA4, Switzerland), which pumped the feeding solution from a temperature controlled storage tank (ISCO FTD 220, Italy) to the beds.

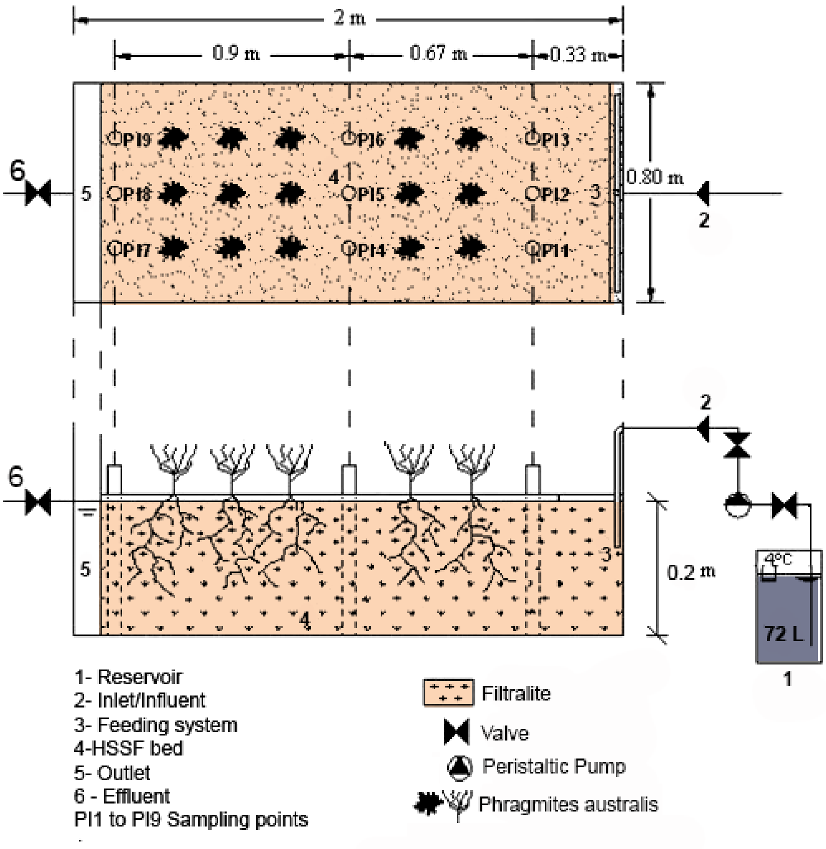


Figure 5.1 Laboratory set-up

5.2.2. Feeding solution

Synthetic wastewater was used in the experiments, which included an organic carbon source (sodium acetate solution), a nitrogen source (ammonia chloride) and a mineral source as proposed by Albuquerque *et al.* (2009b). All the solutions were prepared as concentrated ones according to the following composition: buffer solution ((8.50 g KH_2PO_4 + 21.75 g K_2HPO_4 + 33.40 g $Na_2HPO_4.7H_2O$ + 1.70 g NH_4Cl) L^{-1}), magnesium sulphate solution ((22.50 g $MgSO_4.7H_2O$) L^{-1}), calcium chloride solution (36.43 g $CaCl_2.2H_2O$ L^{-1}), iron chloride solution (0.25 g $FeCl_3.6H_2O$ L^{-1}), oligoelements solution ()0.04 g $MnSO_4.4H_2O$ + 0.06 g H_3BO_3 + 0.04 g $ZnSO_2.7H_2O$ + 0.032 g

$(NH_4)_6.Mo_7O_{24}.4H_2O$ + 0.0555 g EDTA + 0.0445 g $FeCl_3.6H_2O$) L^{-1}), sodium acetate solution (113.4 g $C_2H_3O_2Na.3H_2O$ L^{-1}, which gave 50 g COD L^{-1}), ammonia chloride solution (76.41 g NH_4Cl L^{-1}, which gave 20 g N L^{-1}).

The desired concentrations of COD and NH_4-N for the experiments were obtained by diluting the concentrated solutions of sodium acetate and ammonia chloride. The feeding solution was kept in the storage tank (72 L) at constant temperature (4±0.2 ºC) and was changed each 3 days.

5.2.3. Operating conditions

The mesocosms were continuously fed during 50 days (8 weeks) for a flow rate of 1 $L.h^{-1}$ (HLR of approximately 3.6 $cm.d^{-1}$ which corresponded to an HRT of approximately 6 days divided by the effective bed volume). Two sets of experiments were carried out: Phase I at COD concentrations of ~300 $mg.L^{-1}$ (OLR ~10 $gCOD.m^{-2}.d^{-1}$) and NH_4^+-N concentrations of ~30 $mg.L^{-1}$ (NLR ~1 gNH_4^+-N. $m^{-2}.d^{-1}$), followed by Phase II at COD concentrations of ~500 $mg.L^{-1}$ (OLR ~20 gCOD $m^{-2}.d^{-1}$) and NH_4^+-N concentrations of ~50 $mg.L^{-1}$ (NLR ~2 gNH_4^+-N $m^{-2}.d^{-1}$). In any case, the COD/N ratio was ~10 (corresponding to a C/N ratio of ~4).

Weekly water samples were collected from the influent and at the sampling points PI2, PI5 and PI8 (the samples from PI8 were considered as effluent samples, since it was located close to the discharge point as also admitted in Albuquerque et al. (2009b) to analyse the temperature, pH, dissolved oxygen (DO), COD, NH_4^+-N, nitrite nitrogen (NO_2^--N) and nitrate nitrogen (NO_3^--N). The temperature in the room was 20±0.4 ºC.

5.2.4. Analytical methods

The measurements of temperature, pH and DO were carried out through probes Sentix 41 and CellOx 325 connected to a Multi 340i meter (WTW, Germany). The COD was evaluated by closed reflux digestion and utilizing titrimetric method (APHA-AWWA-WEF 1999). Concentrations of NH_4^+-N, NO_2^--N and NO_3^--N were obtained using the cuvette-tests LCK 302 (47-130 $mgNH_4^+$-$N.L^{-1}$), LCK 303 (2-47 mg NH_4^+-$N.L^{-1}$), LCK 342 (0.6–6 mg NO_2^--$N.L^{-1}$), LCK 339 (0.23-13.5 mg NO_3^--$N.L^{-1}$) and LCK 340 (5-35 mg NO_3^--$N.L^{-1}$), following the standards DIN 38406-E 5-1 (ammonia), DIN 38405 D10 (nitrite) and DIN 38405-9 (nitrate), and the CADAS 50 spectrophotometer UV-Vis (HACH LANGE, Germany).

Statistical analysis was executed through one-way ANOVA (analysis of variance), considering a significance level of $P<0.05$. The mass removal rates (in $g.m^{-2}.d^{-1}$) were calculated through the difference of concentrations in the influent and the effluent (point PI8) times the flow-rate divided by the effective area of each bed.

5.3 Results and discussion

The results of the experiments for each bed are shown in Table 5.1.

Table 5.1 Results for unplanted and planted lab-scale CW under lower and higher loading rates

Parameters	Lower loading rate				Higher loading rate			
	Unplanted		Planted		Unplanted		Planted	
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
Temperature (ºC)	19.2 ± 0.54 (18.4 - 19.9)	21.5 ± 1.14 (20.0 - 23.7)	20.4 ± 1.46 (18.3 - 22.0)	21.4 ± 1.38 (19.4 - 22.6)	16.1 ± 1.29 (15.0 - 18.4)	19.0 ± 0.94 (17.6 - 20.8)	15.7 ± 1.43 (13.9 - 18.9)	19.0 ± 0.78 (17.8 - 20.6)
pH	7.3 ± 0.1 (7.2 - 7.5)	8.1 ± 0.1 (7.9 - 8.4)	7.2 ± 0.1 (7.1 - 7.3)	8.1 ± 0.4 (7.5 - 8.4)	7.1 ± 0.2 (6.9 - 7.5)	7.9 ± 0.2 (7.7 - 8.4)	7.2 ± 0.2 (7.0 - 7.5)	7.6 ± 0.4 (7.0 - 8.5)
DO (mg L^{-1})	6.6 ± 0.5 (5.7 - 7.4)	0.2 ± 0.1 (0.1 - 0.2)	5.7 ± 1.5 (1.3 - 4.4)	0.3 ± 0.2 (0.0 - 0.5)	4.7 ± 0.5 (3.9 - 5.1)	0.4 ± 0.2 (0.2 - 0.7)	5.7 ± 1.2 (3.3 - 7.6)	0.3 ± 0.2 (0.2 - 0.6)
COD (mg L^{-1})	311.2 ± 18.7 (292.0 - 345.0)	191.5 ± 6.5 (185.0 - 202.0)	315.9 ± 38.8 (279.5 - 369.0)	93.8 ± 36.1 (59.4 - 152.0)	511.5 ± 23.3 (471.5 - 548.0)	289.5 ± 37.3 (218.0 - 335.0)	517.0 ± 20.4 (481.0 - 550.0)	133.4 ± 84.6 (18.1 - 250.0)
Removal Efficiency (%)	38.50 ± 1.80		73.90 ± 10.50		44.40 ± 7.10		75.40 ± 16.20	
OLR (g m^{-2} d^{-1})	11.85 ± 0.71		11.16 ± 1.37		19.75 ± 0.97		18.74 ± 0.64	
r_{COD} (g m^{-2} d^{-1})	4.59 ± 0.48		8.88 ± 1.95		8.39 ± 1.44		13.12 ± 2.51	
NH_4-N (mg L^{-1})	26.7 ± 2.2 (24.0 - 29.9)	17.6 ± 2.5 (15.0 - 21.4)	36.3 ± 5.8 (27.3 - 40.5)	14.8 ± 9.5 (4.1 - 24.1)	50.0 ± 3.4 (44.2 - 54.4)	34.0 ± 4.4 (28.8 - 40.8)	53.0 ± 5.1 (44.6 - 62.3)	27.9 ± 10.5 (9.2 - 42.2)
Removal Efficiency (%)	33.9 ± 8.9		59.3 ± 25.6		33.0 ± 6.0		47.8 ± 17.5	
NLR (g m^{-2} d^{-1})	1.01 ± 0.08		1.28 ± 0.21		1.87 ± 0.13		1.86 ± 0.20	
r_{NH4N} (g m^{-2} d^{-1})	0.35 ± 0.10		0.76 ± 0.34		0.62 ± 0.11		1 ± 0.24	
NO_2-N (mg L^{-1})	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6
NO_3-N (mg L^{-1})	< 0.23	< 0.23	< 0.23	0.5 ± 1.0	< 0.23	< 0.23	< 0.23	< 0.23
COD/N	11.7	10.9	8.7	6.3	10.2	8.3	9.8	4.8

Note: Average, standard deviations and minimum and maximum values (in brackets) for 5 samples in L2 (Phase I), 8 samples in the other experiments. Most of the NO_2-N and NO_3-N concentrations were below the detection limit of the analytical methods (<0.6 mg NO_2-N L^{-1} and <0.23 mg NO_3-N L^{-1}
.

5.3.1 Temperature, pH and dissolved oxygen

The temperature profiles show a slight increase along the beds for all the experiments, but no significant ($p>0.05$) differences were noticed for the planted and unplanted beds. The temperature increase along the bed was probably caused by the increase of microbial activity related to biodegradation processes. Therefore, the effect of the temperature on the removal of COD or N was neglected.

The average influent pH was ~7.2, increasing approximately one unit in the effluent in all the experiments. Considering that the acetate oxidation produces alkalinity and that the ammonia oxidation through nitrification consumes alkalinity, the alkalinity production due to acetate oxidation was greater than its consumption by nitrification. There was no detection of nitrate or nitrite at the sampling points, even if the oxidation of ammonia occurred in both beds for the different loads. Therefore, simultaneous reduction of nitrate and nitrite through denitrification has occurred in all the sections and could have also contributed to the pH increase, since this process produces alkalinity as also observed in Bialowiec *et al*. (2011) and Bialowiec *et al*. (2012a).

The average effluent pH decreased with the increase of the NLR, which may be explained by the increase on nitrification as also observed in a previous study with the same laboratory mesocosm (Albuquerque *et al.* 2009b). The values observed in this study are, however, within the recommended range of values ($4.0<pH<9.5$) that allow a good activity of heterotrophic bacteria, denitrifiers and autotrophic nitrifiers (Kadlec & Wallace 2008).

The average DO also decreased along the beds in all the experiments, with a drop to ~0.5 $mg.L^{-1}$ in the first measuring point (PI2), remaining close to zero in the other measuring points (PI5 and PI8) in both beds. This fast drop of DO immediately after the feeding point is explained by the aerobic removal of COD and NH_4-N (nitrification) in the first section of the beds (Inlet-PI2) due to high oxygen demand.

5.3.2 Effect of loadings and plants on COD removal

COD concentrations decreased along both planted and unplanted beds, with a bigger drop in the first section (inlet-PI2), and then continued to decrease slowly until the last sampling point (PI8). The planted bed showed higher removal than the unplanted bed, which was statistically very significant ($P<0.05$). For the lower OLR (Phase I) the maximum COD removal efficiency (RE) was 87.6%, whilst for the higher OLR (Phase II) the maximum value was 96.2%, in both cases for the planted bed.

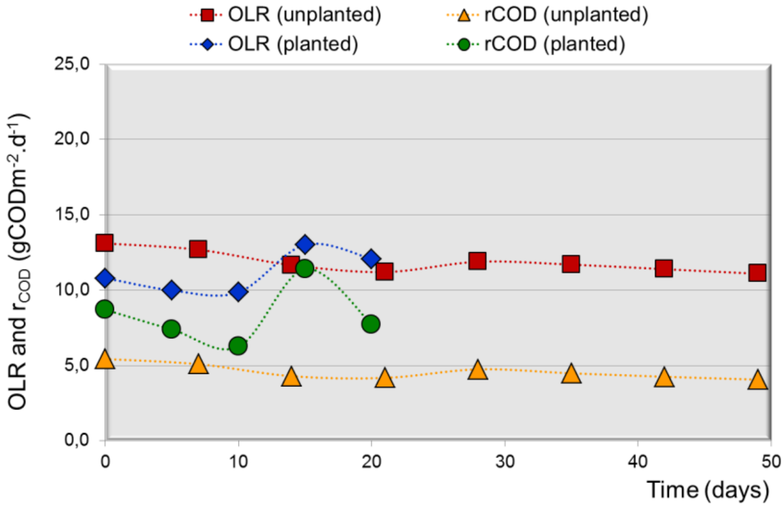
For the lower OLR, after 3-week of operation period the *steady-state* conditions were observed at the unplanted bed with an average RE of 39.5% for COD (the average RE for all the experiments was 38.5%). On the contrary, COD removal in the planted bed showed more variability, with average RE of 72.6% in the first 2 weeks, 57% after 4 weeks and 73.9% for the overall experiments. For the higher OLR, a similar trend was observed for both beds with the effluent COD concentrations stabilizing after 2 weeks for the unplanted bed (the average RE for all the experiments was 44.4%) and increasing over in time for the planted bed (the average RE for all the experiments was 75.4%). The fluctuation in COD removal in the planted bed would mean that the development of plants and roots over time affected the bed dynamics, which influenced organic matter removal.

The lower RE observed in the unplanted bed was probably due to the lower availability of oxygen, which is necessary for the microbial degradation of organics. The difference in COD removal observed in both beds is consistent with some works developed in HSSF, which have pointed out that plants may play an important role in the removal of organics (*e.g.* Tanner, 2001; Kadlec & Wallace, 2008; Albuquerque *et al*., 2009b), but inconsistent with others where it was detected the interference of organic exudates (released by roots) on COD removal (e.g. Hunter *et al*. 2001; Bialowiec *et al*., 2012a).

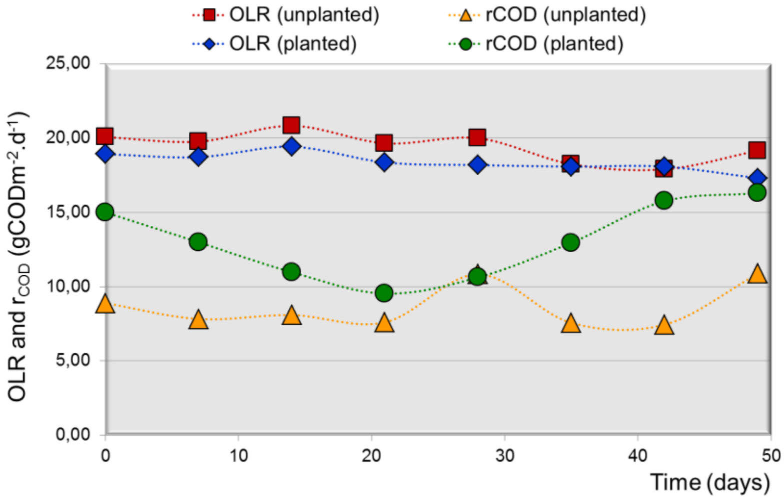
The results for the planted bed (L2) are within the range of the values found for COD removal (70% to 90%) in LECA-based HSSF working under similar operating conditions (van Deun & van Dyck, 2008; Albuquerque *et al*., 2009b; Bialowiec *et al*., 2012b).

At the lower OLR the average mass removal rate for COD (r_{COD}) was lower in the unplanted bed (4.6 $gm^{-2}.d^{-1}$), increasing to 8.9 g $m^{-2}.d^{-1}$ in the planted bed (93.4% more) as shown in Figure 5.2a). At the higher OLR the average r_{COD} ranged from 8.4 $g.m^{-2}.d^{-1}$ (unplanted bed) to 13.1 $gm^{-2}.d^{-1}$ (planted bed), *i.e.* 56% more (Figure 5.2b).

Removal rates normally increased as the OLR increased with a maximum value of 16.3 $gCOD.m^{-2}.d^{-1}$ for the maximum OLR (19.8 $gCOD.m^{-2}.d^{-1}$). Vymazal & Kröpfelová (2008), reports average r_{COD} in gravel-based HSSF-CW of 8.5 $gCOD.m^{-2}.d^{-1}$ which equals the average value observed in this study for the planted bed at low OLR and for the unplanted bed at the higher OLR. Therefore, regardless both the presence of vegetation and the OLR, Filtralite aggregates seems to improve the removal of organic matter in comparison with gravel aggregates.



a)



b)

Figure 5.2 COD removal rates over time for the lower OLR (a) and higher OLR (b) in the planted and unplanted beds.

Regardless of the OLR, for the unplanted bed, more than 75% of the COD was removed in the first section (Influent-PI2), whilst for the planted bed the removal of COD was better distributed along its length (42.5% to 50.3% in section Influent-PI2, 22% to 30.4% in section PI2-PI5 and 19.3% to 35.5 % in section PI5-PI8).

In the first section, COD removal seems to have been associated mainly with the aerobic oxidation of acetate carried out by a heterotrophic biofilm since DO concentrations were higher and organic carbon was mainly soluble (acetate). In the remaining sections (PI2-PI5 and PI5-PI8), since the bed was oxygen-limited, COD removal was associated with the oxidation of acetate and other organic material released by roots (exudates) through anaerobic pathways (fermentation and denitrification), and also due to aerobic oxidation in the rhizosphere where oxygen was released by roots. r_{COD} showed a positive linear correlation with the lower OLR for the unplanted (R^2= 0.93) and planted (R^2= 0.93) beds (Figure 5.3) with statistical significance (p< *0.05*), but no significant linear correlation was found for the higher OLR (R^2 < 0.19 in both beds) and, therefore, this data is not shown as a figure. Therefore, regardless of the presence of plants, COD loads influence the respective removal rates, for OLR from 10 to 13 gCOD $m^{-2}.d^{-1}$.

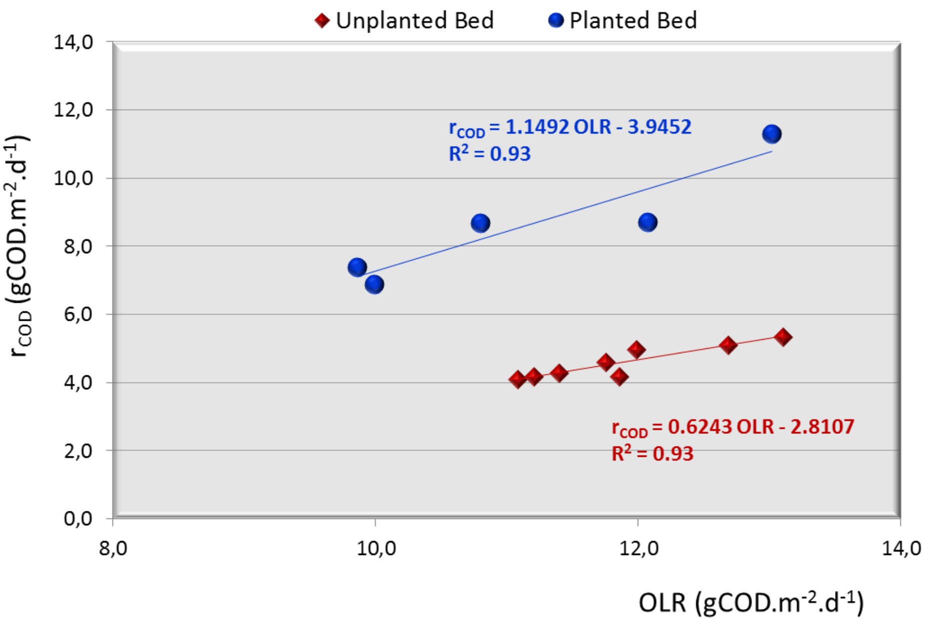


Figure 5.3 Relationship between OLR and r_{COD} in unplanted and planted beds, for the lower load (Phase I).

It seems that planted HSSF improved COD removal due to the combination of mechanisms favoured by plant growth. The growth of rhizomes and roots provide a large surface area and medium for microbial attachment and growth, thus increasing the removal of COD through microbiological pathways. It is also recognized that plants have been linked to the strong redox gradients by releasing oxygen from their roots into the rhizosphere and thereby stimulating aerobic decomposition and growth of nitrifying bacteria (Randerson *et al.* 2010; Bialowiec *et al.* 2011, 2012a).

Therefore, high OLR seem to favour the activity of heterotrophic microorganism and consequently increase COD removal, because at high OLR more organic substrates were supplied, which resulted in high heterotrophic production rates as also observed in the work of Wendong *et al*. (2007).

5.3.3 Effect of loadings and plants on NH_4-N removal

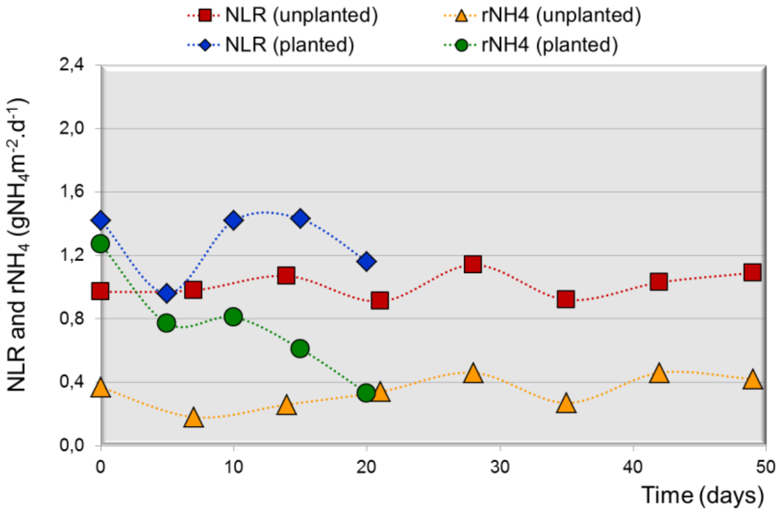
The planted bed presented low effluent NH_4^+-N concentrations, suggesting that plants significantly increased the removal of ammonia. When the NLR was doubled (Phase II), the NH_4^+-N concentrations in the effluent also increased in both beds. Nitrite and nitrate were not detected in both phases (Table 5.1.). This circumstance suggests that all nitrate and nitrite formed through nitrification was removed either by denitrification in anoxic zones or was uptake by plants, as was also observed by Albuquerque *et al*. (2009b) and Bialowiec *et al.* (2012b).

For the lower NLR, NH_4^+-N concentrations slightly decreased along the unplanted bed with overall RE of 23.8% after 2 weeks, 37.5% after 4 weeks and 38.2% after 8 weeks. On the contrary, NH_4^+-N removal in the planted bed showed a high variability with a RE of 79.4% in the first 2 weeks, 43% after 4 weeks and an average of 59.3% for the overall experiments. This fluctuation in ammonia removal could be explained by the variation of DO in the rhizosphere, but also due to organic exudates by roots, which normally include organic nitrogen (Pinton *et al*. 2007; Bialowiec *et al.* 2012a).

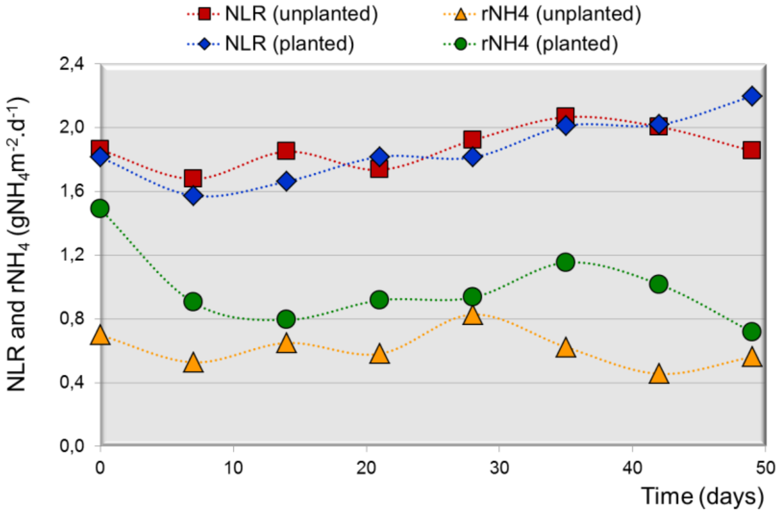
Organic nitrogen may be hydrolysed to ammonia, thus increasing the concentrations of this inorganic nitrogen form in the rhizosphere. Therefore, the oxidation of ammonia occurred faster when the ionized form was available and increased again after a few days due to the hydrolysis of organic nitrogen compounds, presumably released by the plants as observed in the studies of Bialowiec *et al.* (2012a, 2012b). Similar results were observed for the higher NLR. NH_4^+-N loss by volatilization was negligible, since only occurred at pH above 9 (Kadlec & Wallace 2008).

For the unplanted bed the average RE was 33.9% and 33% for the lower and higher NLR, respectively, whilst for the planted bed the average RE were higher (59.3% and 47.8%, respectively). These last values are higher than those found in planted mesocosms operating under similar conditions, which reported RE between 30% and 54% (Hunter *et al.* 2001; Sun & Austin 2007; Cheng *et al*. 2011). However, Mander *et al*., (2000) found high variations in ammonia RE (12% to 85%) for planted HSSF, suggesting that there is a higher density and activity of nitrifying biomass in the planted systems as also observed by Stecher & Weaver (2003).

The planted bed showed significantly higher ammonia mass removal (r_{NH4}) than the unplanted bed as shown in Figure 5.4. The average of r_{NH4} in the unplanted bed was 0.35 gNH_4^+-$N.m^{-2}.d^{-1}$, increasing to 0.76 gNH_4^+-$N.m^{-2}.d^{-1}$ in the planted bed for the lower NLR. For the higher NLR the increase was from 0.62 gNH_4^+-$N.m^{-2}.d^{-1}$ to 1 gNH_4^+-$N.m^{-2}.d^{-1}$. The removal rates observed in the planted bed are lower than the ones found by Vilpas *et al.* (2005) in pilot beds treating domestic wastewater, between 1.8 and 6.1 gNH_4^+-$N.m^{-2}.d^{-1}$, but higher than the rates observed by Kuschk *et al*. (2003) and Scholz (2006) in wetland mesocosms (0.26 to 0.6 gNH_4^+-$N.m^{-2}.d^{-1}$ and 0.23 gNH_4^+-$N.m^{-2}.d^{-1}$, respectively).



a)



b)

Figure 5.4 NH4-N removal rates, over time, for the lower NLR (a) and higher NLR (b) in the planted and unplanted beds.

Plants also have an important role in releasing oxygen into the rhizosphere, therefore promoting the development of nitrifiers and aiding in nitrification. Some studies indicate that an aerobic microzone could be present very close to the root surfaces (Armstrong & Armstrong, 2005; Bialowiec *et al.*, 2011), enhancing aerobic microbial mechanisms that contribute to organic and ammonia oxidation, which may be a significant contribution to the overall removal of those compounds.

Although it was already proved that reeds oxygenate microsites close to the roots (Armstrong & Armstrong, 2005), making the DO available for rapid microbial uptake in these areas, there is still insufficient surplus oxygen to oxygenate the remainder of the bed. In fact, the average DO concentration within the two beds and at the outlet was always lower than 0.5 $mg.L^{-1}$, indicating the prevalence of anaerobic/anoxic conditions, which are characteristic of these beds.

When compared to organic matter, ammonia is more difficult to remove as nitrifiers are autotrophic microorganisms that have a slow respiration rate and stoichiometrically require 4.57 mg per mg NH_4^+-N removed (full nitrification) and 1.71 mg per mg NH_4^+-N removed (partial nitrification) (Paredes *et al*. 2007; Kadlec & Wallace 2009). However, Sun *et al*. (2003) reported that the reduction of ammonia in a down flow reed bed was not balanced by increases in nitrite and nitrate contents in the influent.

When observing the DO measured at the inlet point and the ammonia removed throughout the bed in all experiments it seems that there was not enough oxygen to justify the removal either by full nitrification (oxidation to nitrate) or partial nitrification (oxidation to nitrite). An oxygen balance based on the stoichiometric factors referred above shows that the ammonia removed in both phases in both beds cannot be solely explained by nitrification since it results in a deficit of oxygen.

The values are so high that they cannot be explained by additional atmospheric oxygen diffusion into the bed. It seems, therefore, unlikely that there was sufficient oxygen flux to drive the apparent ammonia removal rates observed in the bed through conventional nitrification. In other words, it can be assumed that other non-conventional ammonia removal pathways may have been present. The adsorption of ammonia, nitrite and nitrate in the LECA could also be neglected as proved before through batch adsorption tests (Albuquerque *et al*., 2009b).

Nitrogen removal in CW has mostly been assumed to be a result of the combination of nitrification-denitrification, plant uptake, assimilation by biomass, precipitation and sedimentation (Vymazal, 2007; Kadlec & Wallace, 2008). However, newly discovered pathways such as anaerobic ammonia oxidation and heterotrophic nitrification (Paredes *et al.*, 2007) could have potential significance in its loss as observed in the works of Sun & Austin (2007), Tao & Wang (2009) and Albuquerque *et al*. (2009b). Therefore, the loss of ammonia might be a result of a combination of different processes, including conventional and non-conventional pathways of the nitrogen cycle as also observed in other anaerobic biofilm rectors (Paredes *et al*., 2007; Albuquerque *et al.* 2009c).

Regardless of the presence of plants and the variation in NLR, a significant linear correlation between NLR and r_{NH4} (R^2 <0.25 and $p>0.05$) was not observed. Therefore, it seems that the NLR was not influenced by the respective removal rates for loads between 1 and 2 gNH_4^+-$N.m^{-2}.d^{-1}$.

Therefore the presence of plants and the increase in both OLR (from ~10 to ~21 $gCOD.m^{-2}.d^{-1}$) and NLR (from 1 to 2 gNH_4^+-$N.m^{-2}.d^{-1}$) had a significant impact on the removal of COD and NH_4^+-N as shown in Figure 5.5.

Phragmites australis requires nutrients for growth and reproduction and ammonia uptake can range between 0.03 to 0.16 $g.m^{-2}d^{-1}$ (Vymazal & Kröpfelová 2008), which means a 10% to 15%

contribution to ammonia removal (Vymazal 2007; Kadlec & Wallace 2009). On the other hand, as roots and rhizomes get more developed, the surface area occupied by nitrifying biofilm also increases.

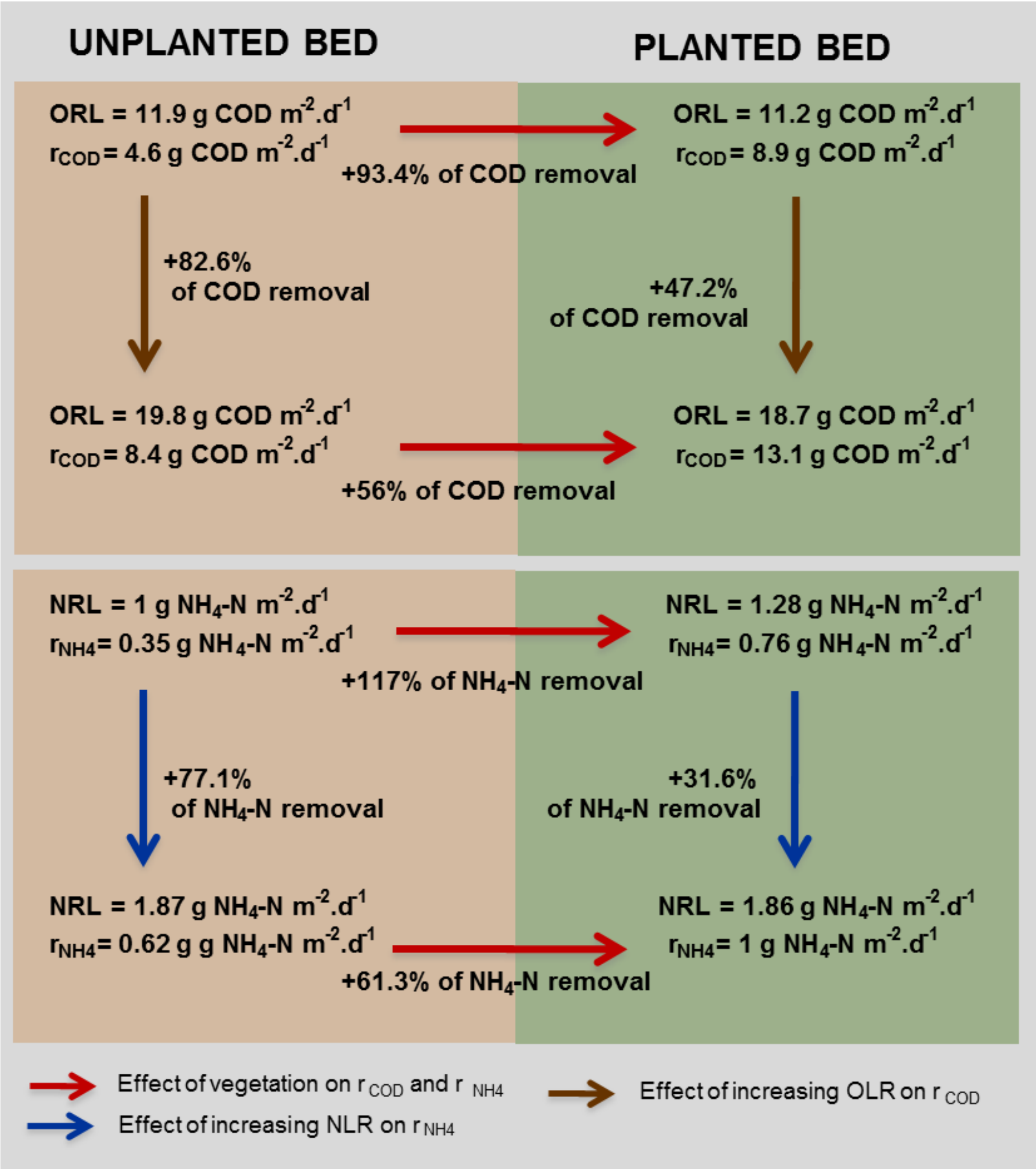


Figure 5.5 Effect of plants and loads on the removal of COD and NH_4-N.

5.4. Conclusions

Data from planted and unplanted HSSW suggests that regardless of the applied loads the presence of plants provides a higher removal of COD and NH_4–N. COD removal was influenced by OLR between 10 and 13 $m^{-2}.d^{-1}$, but no linear relationship was found for higher OLR, ors for nitrogen. LECA is a very suitable material for CW technology since its both higher porosity and specific surface area allow the fast growth of plants, as well as rapid adhesion and development of biofilm.

Acknowledgement

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6. Oxygen transfer capacity in the rhizosphere of *Phragmites australis*[5]

Submitted to Ecological Engineering (October 2016)

Abstract

Constructed wetland beds are considered anaerobic bioreactors. However, oxygen release by roots and rhizomes of plants may contribute for creating an oxidizing micro-environment, which will provide conditions for the development of oxidizing processes such as anoxic respiration or nitrification. Eight experiments with Phragmites australis were running for evaluating the oxygen transfer capacity (OTC) in the root zone, four running for one day and the other four for up to five days. Results show an OTC production ranging from 0.03 $gO_2.m^{-2}.d^{-1}$ to 1.53 $gO_2.m^{-2}.d^{-1}$, which are enough for creating oxidizing conditions close the roots and rhizomes. The DO patterns show clear fluctuations of oxygen through light and dark periods, which also influenced the patterns of ORP.

Keywords: Constructed wetlands; Horizontal subsurface flow; Phragmites australis; rhizosphere, oxygen transfer capacity

[5] Mesquita M. C., Albuquerque A., Nogueira R., Amaral L., 2016 “Oxygen transfer capacity in the rhizosphere of *Phragmites australis*” Ecological Engineering (submitted October 2016)

6.1. Introduction

In the last decade, artificial constructed wetlands (CWs) have been used for domestic, industrial, landfilling leachate and agricultural run-off wastewater treatment with good results even for transient load conditions (Kadlec *et al.,* 2000; Vymazal & Kröpfelová, 2008; Albuquerque *et al*., 2009; Wu *et al.*, 2010). These systems have the advantage of being relative simple and easy to operate and maintain and are eco-friendly and low-cost technologies. There are various technological designs, but the most widely used is the subsurface flow constructed wetlands (SSF-CWs), which consist of a planted porous media bed through which wastewater flows either vertically or horizontally (Kadlec & Wallace, 2009). The most used wetland plants are common reeds (*Phragmites australis*), bulrushes (*Scripus* spp.), cattails (*Typha* spp.), and rushes (*Juncus* spp.) (Taylor *et al*., 2011; Vymazal, 2011; Saeed & Sun, 2012).

Wetland plants have several functions related to the treatment processes, namely removing nutrients though its root uptake (Vymazal, 2011), contributing for the dynamics of water loss, mainly by evapotranspiration (ET) (Headley *et al*., 2012), providing a surface for potential attachment for bacteria to achieve the nitrification and the aerobic degradation of organic matter, particularly the soluble part (Vymazal, 2011). Additionally wetland plants induce biochemical changes in the rhizosphere such as pH, oxidation-reduction potential (ORP), and dissolved oxygen concentration (DO), which in turn improves the degradation mechanisms and increases nitrification (Bezbaruah & Zhang, 2004).

The exudates release by roots can be a labile carbon source for enhancing denitrification and for stimulating microbial growth and activity in the rhizosphere. They are low-molecular weight organic compounds (e.g. polysaccharides, amino acids, sugars, organic acids) that corresponded to ~5 to 25% of carbon photosynthetic fixed (Bezbaruah & Zhang, 2004; Kadlec & Knight, 1996; Taylor et al., 2011).

CWs are considered as anaerobic bioreactors. However, the oxygen release by plants through rhizomes and roots can create oxidizing conditions around these plants parts, which is rapidly consumed by nitrification and anoxic respiration. The oxygen required for nitrification and other aerobic microbial processes, might diffuse into the plant tissue from the atmosphere, and enter into theroot zone through the porous stems and roots of emergent plant (Armstrong *et al*., 2000).

Wetland plants are physiological adapted to survival in waterlogged areas because they have an efficient atmospheric oxygen (O_2) transport from above-ground tissues by diffusive and/or convective mechanisms via gas channel tissues (aerenchyma) inside the plants down to the root system to provide the oxygen necessary for cell respiration of roots and rhizomes to provide sufficient energy for growth, maintenance and nutrient uptake (Armstrong & Armstrong, 1990; Wieβner *et al*., 2002; Armstrong & Armstrong, 2005; Colmer, 2003). However, part of the oxygen supplied via aerenchyma to roots and rhizomes in anaerobic media can be radially diffuse into the surrounding medium, process called radial oxygen loss (ROL) (Armstrong *et al*., 1994). This results in the formation of a layer of oxygen around the roots of about 1-3 mm thickness that generate oxidizing microzones, which contributes to promote the growth of autotrophic aerobic bacteria (nitrifiers) and help to oxidize various reducing compounds in the rhizosphere that can be phytotoxic like reduced forms of iron, manganese, sulphide and various organic acids (Armstrong & Armstrong, 2005; Colmer, 2003; Kadlec & Wallace, 2009; Reddy & DeLaune, 2008; Voesenek *et al*., 2006; Wieβner *et al*., 2002).

Arth & Frenzel (2000) observed that while nitrification occurred at a distance of 0-2 mm from the surface around individual rice roots, denitrification occurred at 1.5-5.0 mm. According to Armstrong *et al*. (1994), 30-40% of the oxygen transported via the aerenchyma to the root system is lost to the rhizosphere and released continuously, counterbalancing chemical and biological consumption in the root zone. However, the root zone is limited mostly to an upper layer of 30-40 cm, below which the influence of plants is lower, and anaerobic conditions predominate (Bialowiec *et al*., 2012).

Although vegetation has been reported for contributing to oxygen supply through the root zone, the amount of oxygen transported from the roots into zones under the water table is still under discussion. Reed *et al*. (1995) in a review research reported that the amount of oxygen available from plants roots in CWs can be up to 45 $gO_2.m^{-2}.d^{-1}$. The USEPA (2000) reported values between 0 to 32 $gO_2.m^{-2}.d^{-1}$ and Bezbaruah & Zhang (2004) suggest that plants could release ~0.5 to 11 $gO_2.m^{-2}.d^{-1}$. Mburu *et al*. (2013) referred values of 0.450 g $gO_2.m^{-2}.d^{-1}$ for *Typha* spp, while Wiessner *et al.* (2005) in hydroponic cultures of individual plantlets of *Typha latifolia* achieved oxygen release rates of 0.014 $gO_2.h^{-1}$.

The measurements of redox potential have been used in order to characterize oxidation-reduction conditions of wetland media and seem to be particularly useful for characterizing the onset of reducing conditions in a wetland substrate caused by a lack of oxygen following saturation by water (Fiedler & Summer, 2004; Kayser *et al.*, 2003). According to Kim *et al.* (1999), the redox status has been found to be a good parameter for characterizing the rhizospheric conditions, and also important for the oxygen release behaviour of the macrophytes. Oxygen release depends on to the diurnal cycles of day and night as reported by Benstead & Lloyd (1994), which detected diurnal changes in the concentration of dissolved gases (CH_4, CO_2 and O_2).

The role of plants in the release of oxygen in the rhizosphere of CW still remains unclear and it is important to find out more information about oxygen release capacity by wetland plants and on the conditions influencing this process, because their ability to oxygenate the rhizosphere is essential for enhanced biochemical mechanisms for pollutant removal such as nitrification. On the other hand, according Libelli (2006) monitoring the parameters ORP, DO and pH allows determining the termination points of the nitrification and denitrification processes.

Considering the role of oxygen in the removal of contaminants and because oxygen released from macrophyte roots has often been considered to be the major pathway of oxygen flux to these constructed wetlands, the aims of this study was to provide a better understanding of the oxygen transfer capacity (OTC) around roots of *Phragmites australis* and to investigate the DO fluctuations at the laboratory-scale systems.

6.2. Material and Methods

6.2.1. Experimental setup

The experimental setup included a 1500 mL recipient (13.5 cm in diameter), with 1000 mL of deionized water, sparged with nitrogen gas during 20 minutes for stipping DO before each experiment (Figure 6.1). The end point of sparging was defined for an oxygen saturation below 5% and was measured by an oxygen sensor CellOx 325 connected to a Multi 340i meter (WTW, Germany).

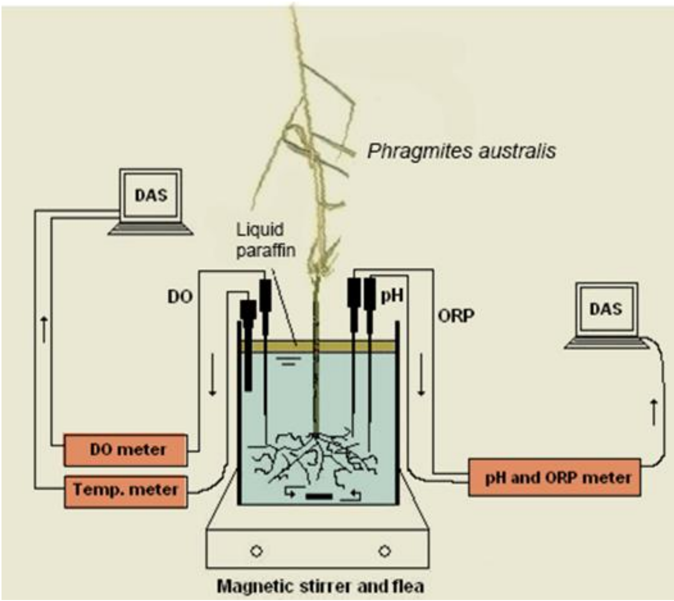

Figure 6.1 Schematic representation of the set-up

Eight *Phragmites australis* plants were used in the study, which were collected from the HSSF-CW mesocosm used in the study of Mesquita *et al.* (2013). The roots of *Phragmites australis* plants were submerged in the water into the central part of the recipient and followed immediately by layering the water surface with one cm of paraffin oil for preventing oxygen diffusion to water from atmosphere.

Therefore, the roots were the only oxygen source within the recipient. Three microelectrodes were used for the measurement of DO (OX-N, 1 mm), ORP (RD-N, 1 mm) and pH (pH-N, 1 mm) of Unisense (Denmark). The OX-N microelectrode was connected to an Oxy-meter (4-channel picoammeter PA2000, Unisense, Denmark) and the RD-N and pH-N microelectrodes were connected to a 4-chanel PHM2010 milivoltmeter (Unisense, Denmark). The temperature was measured through a Sentix41 electrode connected to a Multiline 320i meter (WTW, Germany). Complete mix conditions in the beaker were maintained by a magnetic stirrer (100 rpm, Nahita, UK). The vessel was wrapped in aluminium foil to prevent light penetration into the rhizosphere as suggested by Wieβner et al. (2002). The room air temperature and the water temperature in the beaker were ~20 ^{o}C with natural light.

6.2.2. Experimental plan

Plants numbered with 1, 2, 3 and 4 were used to assess OTC during one day, whilst plants numbered with 5, 6, 7 and 8 were investigated for several days to evaluate the diurnal changes of DO level due to plants activity, and to calculate the OTC changes along time. DO, ORP, temperature and pH were measured instantaneously during all the experiments starting after plants were put into the recipient. However, the three Sensors were connected to two data acquisition systems (DAS) that were programmed to only store measurements each 1 minute. All plants used in the trials were selected in order to present a similar vegetative development. All plants used in the tests were selected in order to be in the same vegetative stage and to put forward similar height and number of leaves.

OTC (mg O_2 L^{-1}.h-^{1}) was calculated according Eq. (6.1) to Eq. (6.4) provide by Randerson *et al.* (2011), which are commonly used in experiments for determining OTC.

$$\mathrm{OTC} = 11.33\ \frac{1}{\Delta T} \ln \frac{D_0}{D_t} \sqrt{\frac{k_{10}}{k_t}} \qquad \text{Eq.6.1}$$

Where, 11.33 is the maximum oxygen saturation (mg L^{-1}) in water at 10 ºC and atmospheric pressure (1013 hPa), ΔT the duration of the measurements (h), D0 the initial deficit in oxygen (CS – C0) (mg.L^{-1}), Dt the deficit in oxygen in time t (CS – Ct) (mg.L^{-1}), C0 the initial oxygen concentration (mg.L^{-1}), CS the maximum oxygen concentration (mg.L^{-1}), Ct the oxygen concentration in time t (mg.L^{-1}) and K10/Kt the coefficient for temperature compensation for temperature 20 ºC (0.784).

ΔT may be calculated from Eq. (6.2) and Eq. (6.3), where tan α angle is the rate of oxygenation.

$$f\left(\log \frac{D_0}{D_t}\right) = \Delta T \qquad \text{Eq. 6.2}$$

$$\mathrm{tg}\alpha = \frac{\left(\log \frac{D_0}{D_t}\right)}{\Delta T} \qquad \text{Eq. 6.3}$$

Therefore, the OTC may be estimated through Eq. (6.4).

$$OTC = 26.1 \, tg\alpha \sqrt{\frac{k_{10}}{k_t}} \qquad \text{Eq. 6.4}$$

The OTC shows the rate of oxygen release to water with initial oxygen concentration, at temperature of ~20 ºC, and under atmospheric pressure. The OTC values can be converted in mg O_2 m^{-2}.day-^{1}, if the surface area of recipient is known.

6.3. Results and Discussion

The results (Table 6.1) show a variation of OTC over time in the eight experiments, with average OTC values of 0.45 $gO_2.m^{-2}.d^{-1}$ (day 1, seven plants), 0.58 $gO_2.m^{-2}.d^{-1}$ (day 2, four plants), 0.29 $gO_2.m^{-2}.d^{-1}$ (day 3, two plants) and 1.53 gO_2. $m^{-2}.d^{-1}$ (day 5, 1 plant). Therefore, the maximum average OTC was observed at the second day of experiments, since there is only one result for day 5. DO variation are presumably associated to the size of the plant and also to oxygen demands of roots and rhizosphere, where can prevail facultative anaerobes microorganisms that are competitive for oxygen molecules, because in reduced media these systems compete for the plant pool of oxygen simultaneously (Armstrong *et al*., 1991; Armstrong *et al*., 1994). Potential redox conditions and microbial oxygen demand are among the main factors that affecting root oxygen release to the rhizosphere (Laskov *et al.*, 2006).

Table 6.1. Oxygen Transfer Capacity Results

Plant No	OTC ($gO_2\ m^{-2}.d^{-1}$)				
	Running days				
	1	2	3	4	5
One day experiments					
1	1,09				
2	0,13				
3	0,20				
4	0,05				
Up to five days experiments					
5	1,11	0,52	b)	b)	b)
6	a)	0,25	0,54	a)	1,53
7	0,05	0,12	0,03	b)	b)
8	0,54	1,43	b)	b)	b)

a) Not possible determining.

b) Plant died.

pH ranged from 6.6 and 7.1 in all experiments, with an average value of 6.8, which are quite stable conditions for plant activity. Average temperature for all experiments was 20.4 ºC. *Phragmites australis* is generally tolerant of temperature variations, but the optimum temperature for growth is between 12 ºC and 23 ºC.

Brix & Schierup (1990) report that *Phragmites australis* can release up to 0.002 $gO_2.m^{-2}.d^{-1}$, these values are in the same range (0.014-0.015 $gO_2.m^{-2}.d^{-1}$) of the ones reported by Ye *et al* (2012) for the same plant species. However, Armstrong *et al.* (1990) and Wu *et al.* (2011) consider that the oxygen released at roots of *Phragmites australis* can be higher (between 4 and 12 $gO_2.m^{-2}.d^{-1}$) depending on the size of the plant and the characteristics of the water, such as salinity and the presence of inhibitory compounds. According to Kadlec & Wallace (2009), the rates of root-zone OTC for emergent species commonly used in treatment wetlands are in the range of 0 to 12 $gO_2.m^{-2}.d^{-1}$, with most in the range of 0.5 to 6 $gO_2\ m^{-2}.d^{-1}$. Therefore, the results of the present study show that *Phragmites australis* can release suitable oxygen in the root zone, which could be suitable for creating oxidizing conditions for enhancing oxidizing mechanisms such as anoxic respiration or nitrification.

A DO peaks was noted in all experiments with one day running as expressed in Figure 6.2 for plant 3, which correspond to the light period (10 *a.m* to 18 *p.m*). In more prolonged measurements of DO concentrations (plants 5 to 8) the observed changes indicated clear diurnal fluctuations of oxygen levels in water, with the several peaks showing the DO pattern during the day period (7 *a.m* to 18 *p.m*) and the lower values showing the dark period (18 *p.m* to 7 *a.m*). Increase of oxygen under lightening conditions and the decrease under darkness were found for all the four plants as it can be seen in Figures 6.3 and 6.4 for plants 6 and 7 with running times of 5 days and 3 days, respectively. Plants influenced differently on oxygen concentration changes during time.

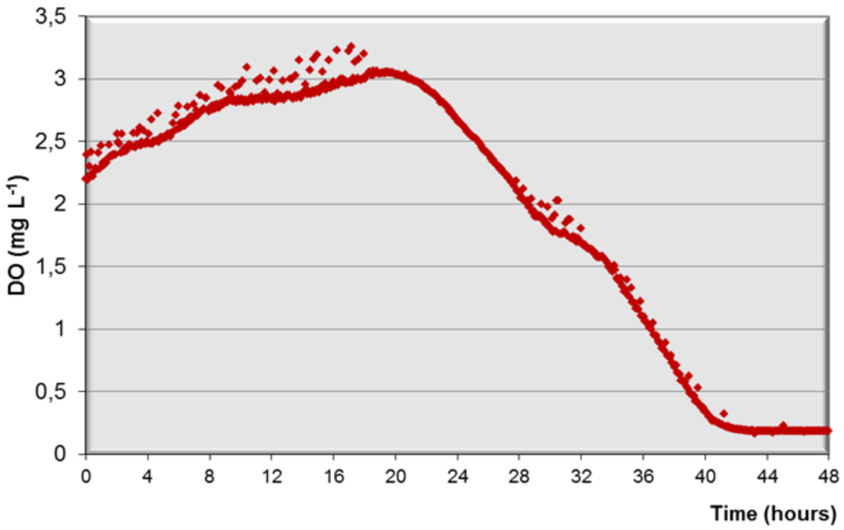

Figure 6.2. Diurnal fluctuations of oxygen concentration during experiment with plant 3 (running time: two day)

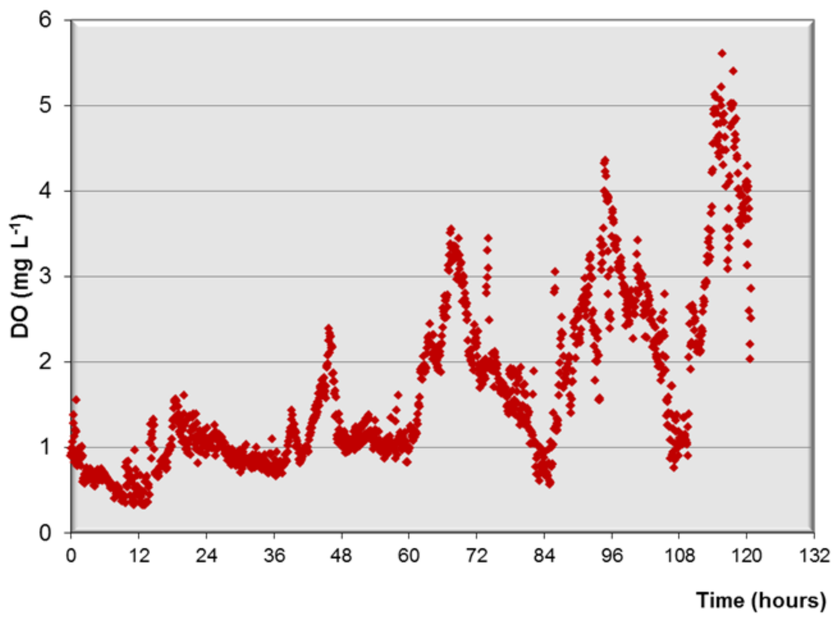

Figure 6.3. Diurnal fluctuations of oxygen concentration during experiment with plant 6 (running time: five days)

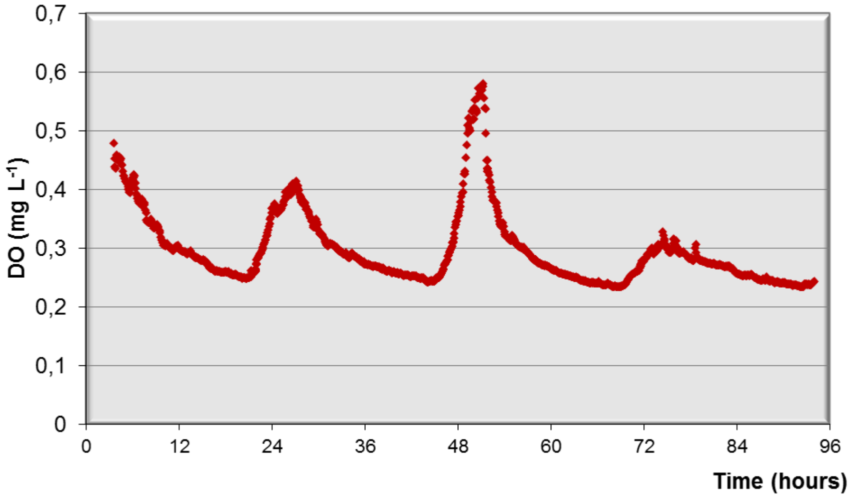

Figure 6.4. Diurnal fluctuations of oxygen concentration during experiment with plant 7 (running time: three days)

Williams *et al.* (2010) found a similar influence of day-night cycles on plants activity in oxygen controlling in the root-zone for willows, using the membrane inlet mass spectrometry (MIMS) technique for monitoring continuously changes of concentrations of dissolved gases (O_2, CO_2, CH_4). In Figure 6.3, the five days experiment, and Figure 6.4, the three days experiment, clearly indicated diurnal cycles of DO concentration with the peaks coinciding with times of day and reducing during the night due to respiratory consumption, with high productivity in the fifth day (1.53 $gO_2.m^{-2}.d^{-1}$) and the second day (0.12 $gO_2.m^{-2}.d^{-1}$), respectively.

Laskov *et al.* (2006) referred that most of oxygen transported to roots and release into surrounding environment has its origin in photosynthetic activity, therefore, the amount of oxygen in the rhizosphere will tend to fluctuate over diurnal periods and varies between seasons (Williams *et al*., 2010). So, there is higher oxygen release in periods of illumination and according Sheppard & Lloyd (2002) even in periods of relatively low light intensity the amount of oxygen released into the rhizosphere can meet the respiratory oxygen demand of the roots and microorganisms in the rhizosphere.

The ORP measurements also show an increasing of the oxic conditions with diurnal growth of DO as it can been seen in Figure 6.5, as an example, for plant 6. Oxygenation of the media surrounding the roots has been shown to increase potential redox, which enable those plants to survive in otherwise anoxic conditions and which is critical in nitrogen fate and microbial activity (Bialowiec *et al*., 2012). It was not possible to get a good ORP registration for plants 1, 5, 6 and 7.

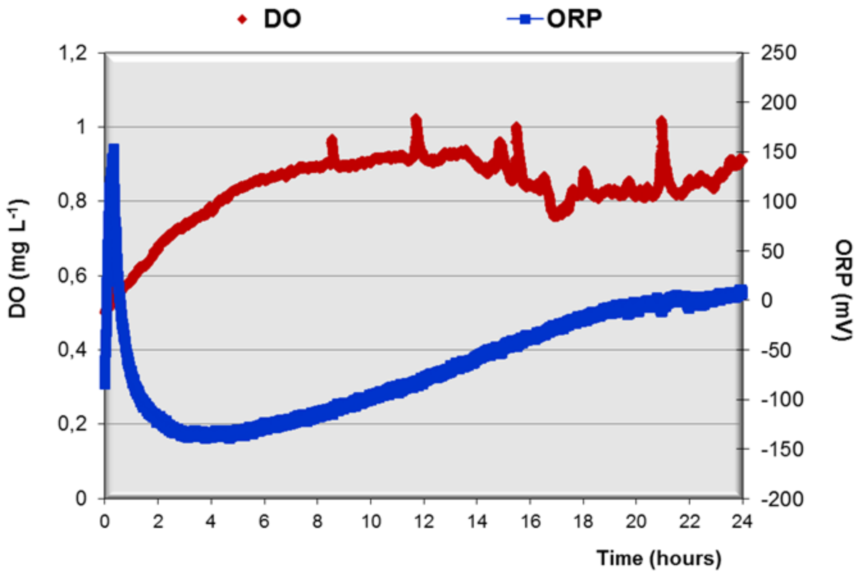

Figure 6.5. Variation of DO and ORP along one day for experiment with plant 6

Table 2.2 shows the average, maximum and minimum values obtained in experiments with plants 2, 3, 4 and 8. The range of values (-152.4 mV (plant 4) to 187.4 mV (plant 8) indicates the prevalence of anaerobic and anoxic environments. However, redox potential in the rhizosphere may change quickly due the amount of oxygen release by roots system that fluctuates over diurnal periods with photosynthesis, light intensity and temperature (Stein & Hook, 2005). For example, Wieβner *et al.* (2002) observed a daily variation in the redox state of *Juncus effusus* rhizosphere under laboratory conditions ranging from -200 mV to +200 mV, driven by daylight.

Table 6.2. Oxidation - Reduction Potential Results

	ORP (mV)		
Plant No	Average	Minimum	Maximum
2	-63,47	-140,14	152,09
3	62,44	14,61	91,07
4	68,15	-152,36	97,18
8	-33,84	-101,54	187,36

According Wieβner *et al*. (2002) the oxidized layer around roots in the rhizosphere can create a gradient of redox potential from capacity ~+500 mV at the root surface to ~-250 mV at 1 to 20 mm away from the root surface. Beyond the plants, redox potential is also affected by water level, activity of microorganisms and by concentrations of electron acceptors (Dušek *et al*., 2008). According to the same author, anaerobic conditions are recognized by a combination of the absence of oxygen and a redox potential lower than +400 mV, while low oxygen conditions are represented by redox values between +400 and -300 mV (Pezeshki & DeLaune, 2012).

Redox potential values below 400 mV indicate activity of denitrifying bacteria and below 100 mV reductions of iron and manganese. When the redox potential value is lower than -100 mV indicates reduction of sulphates and organic matter (fermentation), while below -200 mV indicates activity of methanogenic bacteria (Dušek *et al*., 2008). Most denitrifying bacteria require low redox potential conditions (Eh = +350 to 100 mV) and an organic source (Zhu & Sikora, 1994; Tanner, 1996).

Understanding the processes involved in plant oxygen release in the rhizosphere as well as the oxidizing and reducing capabilities of the root zone are important for understanding the anoxic and oxic removal mechanisms in CW, namely the importance of having non conventional removal mechanism such as anammox or aerobic denitrification and discuseed in the studies of Albuquerque *et al.* (2009).

The larger number of factors that may be effect the amount of oxygen release by plants roots and the difficulty in quantification accurately this release in microscale in situ measurements and/or in laboratory based experiments might explain the disparity in the values observed in this study as well as the ones reported in the literature.

6.4. Conclusions

The batch experiments with Phragmites australis show that this plant can release between 0.03 g $O_2 m^{-2}.d^{-1}$ to 1.53 gO_2 $m^{-2}.d^{-1}$ with higher average production in the second day and which are values suitable for creating oxidizing conditions close the roots and rhizomes for enhancing oxidizing mechanisms such as anoxic respiration or nitrification. A clear pattern for DO was noted in the day-night cycles, which seems to have influenced the ORP in the root zone. This study contributes for a better understanding of the prevalence of conventional and non conventional removal mechanism in the root zone of CW.

Acknowledgements

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6.5. References

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7. Conclusions and future work

Over the last years, CWs have been shown to be environmentally sustainable technology which has successfully treated wastewater from different source, particularly with regard to removal of organic load and suspended solids, characteristics that make this technology fits within the strategic objectives defined in PENSAAR 2020, which aim at ensuring the sustainability of the sector, including the recovery of the investment and operational costs, the protection and improving of the natural ecosystems and the quality of life of population.

Based on the experimental results and conclusions described in each of the previous chapters, it can be drawn some general conclusions for each of the general objectives of the thesis.

Comparing the planted and unplanted units systems, it can be concluded that regardless of the applied loads, planted HSSF-CWs had the highest treatment performance for COD and NH_4^+-N. At lower OLR the average mass removal rate for COD was higher in planted bed when compared to the mass removal rate obtained in the unplanted bed, and it was observed similar behaviour when applied a higher OLR. Results highlight the key role of the plant's presence (*Phragmites australis* – in these experiments) in COD removal and consequently it may conclude that the use of plants in constructed wetlands might be a determinant factor for meeting the limit emission values for discharge of wastewater into surface water bodies.

Many other plants may be used in the CWs and therefore, it would be interesting to do some more laboratory-scale experiment using different macrophytes species and to investigate its effect on the removal and compare their performance.

Influent organic loading rate has proved to influence wetland performance. Results have shown that COD mass removal had a significantly positive linear relationship with mass loading applied for the lower OLR and a slightly significant linear correlation at higher OLR, which would mean that the bed had a satisfactory response to changes in incoming loads. Thus, the results of this study showed that the HSSF-CWs may provide good removal efficiencies of organic matter when exposed to high organic loads, and suggesting that it would also be interesting to develop further laboratory tests that allow clarifying the ability of these beds to support sudden changes in effluent concentrations.

The set of results obtained in this work also suggested that plants significantly increased the removal of NH_4^+-N. The higher ammonia removal in the planted bed is expected due to the ammonia nitrogen uptake by the roots. Higher nitrifying activities are also expected in planted wetlands than in those unplanted systems due to its potential role in releasing oxygen in the rhizosphere and for contributing to increase redox potentials in this zone.

Ammonia removal efficiency decreased at higher loading rates, and this reduction at the highest loading rate might be related to the lack of availability of oxygen, which can limit nitrification process. However, no significant correlation was observed between mass removal rates and ammonia loading rate.

Considering the role of oxygen in the removal of contaminants and because oxygen released through roots has often been considered to be the major pathway of oxygen flux to these constructed wetlands, investigations were performed at the laboratory-scale systems to evaluate oxygen transfer capacity (OTC) and the DO fluctuation by *Phragmites australis*. The results have been shown that this plant can release between 0.03 g O_2 m^{-2}·d^{-1} to 1.53 g O_2 m^{-2}·d^{-1}, with higher average production in the second day, which are values suitable for creating oxidizing conditions close the roots and rhizomes for enhancing oxidizing mechanisms such as anoxic respiration or nitrification. There is diurnal fluctuation of oxygen levels, increasing under lightening conditions and decreasing in the darkness, which seems to have influenced the ORP in the root zone.

Concerning the performance results from the four full-scale HSSF-CWs studied at the interior Central region of Portugal, it was observed lower values of COD and TSS removal efficiencies. The lower values of TSS and COD removal efficiencies might be related with the low surface area per inhabitant which was considered in the design of these systems in Portugal, much lower than conventional values for European countries. These results highlight the need of setup specific regulations for Portuguese design of CWs.

The average effluent concentrations for all quality parameters have always satisfied the standards allowed for discharging in superficial waters for wastewater treatment works above 2000 p.e (Decree-law No. 152 of 19th June) even at fairly short hydraulic retention times. Winter seems to be more critical for wetland performance, as it was expected. So, for TSS and COD, the results obtained suggest that HSSF-CWs can contribute to reduce effectively these parameters in wastewater to levels that are in according to the European and national regulatory standards for discharge.

The evaluation of nitrogen removal performance of the full-scale HSSF-CWs was difficult due to the short period of observation, but it can be generally concluded for the four systems that the performance observed for nitrogenous compound, especially for TN and NH_4^+-N, shows that aerobic conditions are not well maintained within the filter beds and removal of these compounds was much lower than expected. Increase of inorganic nitrogen in the effluent was detected and occasional negative values were obtained for the removal rate in all systems for all nitrogen fractions.

The low removal rate of NH_4^+-N showed that nitrification was not effective and the major reason was probably the inability of HSSF-CWs to nitrify due to the low oxygen content which tends to characterize these systems.

Autumn-winter seemed to be critical for nitrogen removal efficiency, being the warmest period the ones to show the higher removal efficiencies for all nitrogen species. Factors that probably include lower flows rate and HLRs and therefore increased HRTs as well as more favourable temperatures for microorganisms involved in nitrification and denitrification processes, along the intense growth of plants might be contributed to enhance the nitrogen removal in warmer period.

The high evapotranspiration rate at high temperatures during hot and dry summers that characterized the continental Mediterranean climate, probably caused water level drop within the bed that may also have contributed to increase the removal of ammonia nitrogen due high nitrification rates associated to higher bed aeration.

Although systems did not removed significant nitrogen compounds, concentrations of total and ammonium nitrogen at the effluent were generally lower than that required by discharge standards in Portugal and EU.

In addition, results from this study indicate that removal efficiencies were also marked by seasonality for phosphorus compounds (TP and DP) with highest percentage removal occurring in spring-summer period, probably due to the direct uptake of phosphorous by plants since this period also corresponds to the exponential growth phase of macrophytes for the climatic conditions in the region under study. The lowered rates of TP removal in the winter months may be due to a decrease in plant density and also to plant decay that could also potentially be an added source of phosphorous. However, the results obtained evidenced that the concentration of TP in the treated effluent does not always meet the limit established for discharge into waters (Decree-Law 236/98 of 1 August), or the limit set in the Urban Wastewater Directive (271/91/EEC) for sensitive zones.

This thesis aims to contribute to a better understanding of the treatment processes that occur in HSSF-CWs, and it is hoped that this work will serve as a springboard for further research in this field. HSSF-CWs will have to be optimized into the future by ameliorating their operation conditions or by developing appropriate strategies based on local parameters because it is expected that the environmental legislation becomes more stringent in view of maintenance and improvement of aquatic ecosystems. The use of these systems can encourage the reuse of treated wastewater in agriculture which is very important given the scarcity of water in the world. As Portugal is one of the countries where desertification has particular relevance, with about 60% of the territory susceptible to desertification and drought, as a result of our climatic, geological and vegetation cover, such situation determine the necessity and importance of implementing measures that contribute to an appropriate sustainable management of water resources in our country. So, the reuse in agricultural of treated effluent could be one of those measures, which could provide a source of water and nutrients for this activity, which has particular expression in the studied region.

Despite the growing evidence for the importance of macrophytes in treatment wetlands, more studies could be conducted to investigate the effective role of different plant species in removing nitrogen. Some studies suggest that polycultures would result in increasing nutrient removal efficiency in CWs systems due to complementary nutrient uses among plant species. Relation between polycultures systems and wetland performance remains an open question which needs to be further investigation.

As future work, the results of this study open a new question regarding the role of non-conventional pathways of the nitrogen cycle in CWs on the loss of ammonia, which might be a result of a combination of different processes, including conventional and non-conventional pathways. So, further attention should be given at non-conventional processes, particularly to assess the importance of Anaerobic Ammonium Oxidation (Anammox) and heterotrophic nitrification in the transformation and removal of nitrogen and to identify environmental parameters (e.g. pH, temperature and DO conditions) and operational parameters (e.g. hydraulic, organic and nitrogen loading rates, HRT, recirculation) that could influence those processes.

Also nitrogen transformation processes such as dissimilatory nitrate reduction and interactions of nitrogen cycle with other elemental cycles such as sulphur are examples of mechanisms involved in the treatment processes in HSSF-CWs that should be also clarified in order to understand more appropriately the potential interactions between the various components within the constructed wetlands. A complete understanding of these factors may contribute to improve treatment performance of HSSF-CWs regarding the removal of nitrogen compounds.

Future research should also provide additional insight for selecting appropriate plant species by quantifying the oxygen transfer capacity associated with other macrophytes species and in different stages of their life cycle and also evaluated the factors that may influence this capacity, an issue that remains unclear and is also important to the design and to optimize the operation of wastewater treatment by HSSF-CWs.

Findings at full-scale HSSF-CWs studies indicate that these systems appear to be limited in nitrogen removal and, therefore, it would be interesting to study alternative configurations that can optimizing nitrogen wetland performances such as the use of hybrid systems, particularly evaluate the use of a vertical subsurface flow system which appears to assure the availability of oxygen and therefore potentially higher rate of nitrification followed by a HSSF-CW in which predominantly anoxic conditions/anaerobic coupled with a higher concentration of oxidized nitrogen in the influent may enhance the growth of denitrifying microorganisms and thus improving the removal of nitrogen compounds.

Appendix

List of publications

Papers published in peer-reviewed journals

Mesquita, M. C; Albuquerque, A; Amaral, L.; Nogueira, R.. 2013. "Effect of vegetation on the performance of horizontal subsurface flow constructed wetlands with lightweight expanded clay aggregates". In International Journal of Environmental Science and Technology 10(3), 433 - 442. DOI: 10.1007/s13762-012-0119-6.

Papers submitted to peer-reviewed journals

Mesquita M. C., Albuquerque A., Nogueira R., Amaral L., 2016 “Oxygen transfer capacity in the rhizosphere of Phragmites australis” Ecological Engineering (submitted October 2016)

Papers in national journals

Albuquerque, A; **Mesquita,** M.C; Amado, L; Craveiro, R. (2014). “Efeito da variação de carga no desempenho de leitos de macrófitas de escoamento sub-superficial horizontal”. In Indústria & Ambiente 85, 26-29.

Papers in national and international conference proceedings

Mesquita, M.C; Albuquerque, A; Amaral, L.; Nogueira, R. 2016. "*Phosphorus removal from domestic wastewater in horizontal subsurface flow constructed wetland with Phragmites australis*". Apresentação na forma de comunicação oral no 1st Symposium of the FibEnTech Research Unit: Fiber Materials and Environmental Technologies Faculty of Engineering, Organizado pelo C-MADE-Centre of Materials and Building Technologies da UBI, 28-29 January, Covilhã, Portugal.

Mesquita, M.C. (2015). “Utilização dos leitos de macrófitas no tratamento de águas residuais domésticas”. Apresentado na forma de comunicação oral no II Workshop de Processos Industriais, organizado pela Escola Superior Agrária de Castelo Branco, 27 de Maio, Castelo Branco.

Mesquita, M. C; Carreiro, Filipe; Albuquerque, A; Amaral, Leonor; Nogueira, R. (2013). "*Removal indicator microorganisms in subsurface horizontal flow constructed wetland*". Apresentação na forma de poster in 4th International Multidisciplinary Conference on Hydrology and Ecology (HydroEcol 2013), 13-16 May 2013, Rennes, France, In 4th International Multidisciplinary Conference on Hydrology and Ecology, Rennes. (publicado em CD-Rom)

Mesquita, M.C.; Carreiro, F.; Albuquerque, A., Amaral, L.; Nogueira, R. (2013). "Seasonal Performance of a full-scale constructed wetland system for Sarnadas de Rodão (Portugal) domestic wastewater treatment". Apresentação na forma de poster in 5th International Symposium on Wetland Pollutant Dynamics and Control (WETPOL 2013). Actas do 5th International Symposium on Wetland Pollutant Dynamics and Control (WETPOL 2013), 13 a 17 de outubro de 2013, Nantes, França (publicado em CD Rom).

Mesquita, M.C; Carreiro, F; Albuquerque, A., Amaral, L.; Nogueira, R. (2013). "Removal indicator microorganisms in three subsurface horizontal flow constructed wetland treating domestic wastewater in Interior Region of Portugal". Apresentação na forma de comunicação oral in 5th International Symposium on Wetland Pollutant Dynamics and Control (WETPOL 2013). In Actas do 5th International Symposium on Wetland Pollutant Dynamics and Control (WETPOL 2013), 13 a 17 de outubro de 2013, Nantes, França (publicado em CD Rom).

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