Phytoremediation in Portugal: a comparison between plants and different wastewaters

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Todas as correções determinadas pelo júri, e só essas, foram efetuadas.

O Presidente do Júri,

MSc

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Abstract:

Phytoremediation comprises the use of plants, and their associated microorganisms, to reduce the concentrations or the toxic effects of different types of contaminants found in the environment. This technology can be used to treat for example surface water and wastewater involving different processes such, mobilization, stabilization, and elimination of contaminants. Main advantages of this technology are that it is Nature-Based, provides several ecosystem services and, in general, is low cost in terms of implementation, operation and maintenance compared with conventional approaches. Some limitations are related to the area needed for establishment and toxicity effects in plants and microorganisms associated to higher concentrations of pollutants. Constructed and Floating Wetlands are examples of phytoremediation technologies for water treatment. The present study aims to do bibliographic research about phytoremediation applied to wastewater management in Portugal, with special focus on the plant species used. The methodology comprised the search of papers, thesis and dissertations, based on "ScienceDirect" search engine and Portugal University´s online repositories. To complement the bibliographic research, field trips were made to two places of technology implementation: a Floating Wetland Island at a port marina, Porto de Leixões, and a constructed wetland at a tourism house, Paço de Calheiros. This framework intended to gather knowledge on the state of the art concerning phytoremediation in Portugal, types of constructed and Floating Wetlands in use, typologies of wastewaters, operational characteristics and specially plant species considered. As a conclusion it was obtained that for the Constructed Wetlands, up to now 90% of the systems implemented in Portugal are horizontal subsurface flow, using mostly the species *Phragmites australis*, *Typha latifolia*, *Arundo donax*, *Iris pseudacorus* and *Canna inidica* that showed efficiency in remediating mostly industrial and domestic wastewaters. Regarding Floating Islands, 100% of the systems were applied at a pilot scale in harbour areas, using a cork platform. For these technologies, the main challenge was the presence of biofouling. Also, the 5 most used plants were: *Sarcocornia perennis*, *Halimione portulacoides*, *Inula crithmoides,* and *Spartina maritima*. Finally, with the application of a pilot system of a Floating Islands, it was possible to assess how easily this system can be applied.

Keywords: Ecosystem Services, Constructed Wetlands, Macrophytes, Phytoremediation, Floating Wetlands, Water Quality.

Resumo:

A fitorremediação compreende o uso de plantas e microrganismos associados para reduzir as concentrações ou efeitos tóxicos de diferentes tipos de contaminantes que são encontrados no ambiente. Esta tecnologia pode ser utilizada para remediar, por exemplo, águas superficiais e águas residuais, envolvendo diferentes processos, tais como mobilização, estabilização e eliminação de contaminantes. As principais vantagens desta tecnologia são o fato de ser uma solução baseada na natureza, providenciar vários serviços ecossistémicos e, em geral, ser de baixo custo em termos de implementação, operação e manutenção, comparando com as abordagens convencionais. Algumas limitações relacionam-se com a área necessária para a construção dos sistemas e os efeitos de toxicidade nas plantas e microrganismos associados a concentrações mais elevadas de poluentes. As FitoETARS e ilhas flutuantes são exemplos de tecnologias de fitorremediação para o tratamento da água residual. O presente estudo visa fazer uma pesquisa bibliográfica sobre a fitorremediação aplicada à gestão de águas residuais em Portugal, com especial enfoque nas espécies vegetais utilizadas. A metodologia incluiu a pesquisa de artigos, teses e dissertações, com base no motor de pesquisa "ScienceDirect", “Google Scholar” e nos repositórios online da Universidade de Portugal. Para complementar a pesquisa bibliográfica, foram feitas viagens de campo a dois locais de implementação de tecnológica: uma ilha flutuante numa marina portuária, Porto de Leixões, e uma FitoETAR numa casa de turismo, Paço de Calheiros. Este trabalho teve como objetivo reuniir conhecimentos sobre o estado da arte em matéria de fitorremediação em Portugal, tipos de FitoETARs e ilhas flutuantes em uso, tipologias de águas residuais, características operacionais e especialmente espécies vegetais consideradas. Como conclusão, foi obtido que para as FitoETARs, até agora 90% dos sistemas implementados em Portugal são de fluxo horizontal subsuperficial, utilizando principalmente as espécies *Phragmites australis, Typha latifolia, Arundo donax, Iris pseudacorus e Canna inidica* que demonstraram eficiência na remediação de águas residuais sobretudo industriais e domésticas. Em relação às ilhas flutuantes, 100% dos sistemas foram aplicados à escala piloto em zonas portuárias, utilizando uma plataforma de cortiça. Para estas tecnologias, o principal desafio era a presença de biofouling. Além disso, as 5 plantas mais utilizadas foram *Sarcocornia perennis*, *Halimione portulacoides*, *Inula crithmoides*, and *Spartina maritima*. Finalmente, com a aplicação de um sistema piloto de ilhas flutuantes, foi possível avaliar a facilidade de aplicação deste sistema.

Palavras-chave: Serviços de Ecossistemas, FitoETAR, Macrófitas, Fitorremediação, Qualidade da Água.

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1. Introduction

1.1 General background

The current demographic growth and urbanization leads to an increase in waste production and energy consumption. Legislation is often not up to date and adapted to current pollution levels (UNESCO, 2017). There is the need to invest in water treatment alternatives aiming for social, economic, and environmental sustainability. In an overall scenario, it is still necessary the wastewater treatment improvement including the removal of certain pollutants such as metals, drugs, pesticides, dye, hydrocarbons, that come from different sources. In most of the cases, the removal of this pollutants can be expensive, requiring a high level of technical knowledge, trained operators, a steady energy supply, chemicals, and equipment (UNEP, 2015). A way to help to solve this problem can be investing in cheaper secondary treatment that use bioremediation and phytoremediation approaches. Through that, some pollutants can be degraded by biological means, extracted from the water, and transformed from high to low toxicity (Abid, 2020).

Phytoremediation can be applied as secondary or tertiary wastewater treatment. It comprises the use of plants, and their associated microorganisms to reduce the concentrations or toxic effects of different types of pollutants usually found in the environment (UNEP, 2019). It is mostly applied to treat polluted soil, contaminated surface water and wastewater (Yadav et al, 2015). The use of phytoremediation is associated with Nature-Based Solutions (NBS) technologies. Nature-Based Solutions are defined as “solutions that are inspired and supported by nature, which are cost-effective, simultaneously provide environmental, social and economic benefits and help build resilience “(EC, 2019). Some technologies such as Floating Wetlands Islands (FWIs) and Constructed Wetlands (CWs), that are supported by plants and microorganisms, provide several ecosystem services. These systems not only improve water quality but also contribute to climate change mitigation and adaptation, promotion of biodiversity, among several other ecosystem services (Calheiros et al, 2020). In Portugal, the interest in phytoremediation-based technology to treat wastewater started at the end of the 80´s and keep growing nowadays (Calheiros et al,2021). According to Dias et al, (2000), at the end of the 20th century there were almost 76 CWs in operation in Portugal although, based on the bibliographic research that was carried out for this thesis, there are no records that bring together information about the constructed wetland and floating islands systems currently in operation in Portugal.

Understanding exactly which factors interfere in the treatment of water through phytoremediation is essential for a satisfactory result. These factors include the type of plants species, mechanisms and symbiotic associations; the type of flow system to be implemented, taking into consideration the place where the system will be built, the budget and the area available; the type of wastewater to be treated, the pollutants to be removed and finally the expected destination or use of the treated water. All these factors are connected and must be previously analyzed in order to avoid waste of material and to make the process as much sustainable as possible (Rahman et al, 2020).

1.2 Nature-Based Solutions and phytoremediation

The large quantities of CO_2 emitted, and waste generated due to the increased urbanization and industrialization has a big impact on the infrastructure and the impermeability of the landscape, which generally cause the degradation of ecosystems, the absence of green spaces, the climatic vulnerability of settlements and the loss of the life quality (Calheiros et al, 2020b). In order to try to counteract this scenario, governments and entities responsible for the environment, have created targets that describe actions and implementations needed around the world. The 2030 Agenda for Sustainable Development Implementation (UN, 2015) was adopted by all United Nations member states in September 2015. The aim is to recover global economic progress with social justice and conserving the natural resources. For that, 17 Sustainable Development Goals were settled. A consequence of this Agenda was the creation of the new urban agenda, created by UN-habitat's that aims a better quality of life for all in an urbanizing world. The document sets advise to countries on where to put efforts towards sustainable urban development, being the integration of NBS one of them (Calheiros et al, 2020b).

There are several benefits that arise with the implementation of NBS allowing for a better quality of life and wellbeing of the population. Some examples are the increase and development of sustainable urbanization, restoration of degraded ecosystems, support to climate change adaptation and mitigation through the improvement of risk management and resilience in cities (EC, 2015). Therefore, by allowing the growth and dissemination of plant species in urban spaces, thus also increasing biodiversity, it is possible to obtain as a consequence better air and water quality, mitigation of problems related with floods and droughts and reduction of the effects generated by global warming. The idea is that by complementing some grey infrastructures with green infrastructures, it is possible to reach new benefits for people and for nature conservation (WWDR, 2018). Some of the benefits of using NBS are depicted in Figure 1.

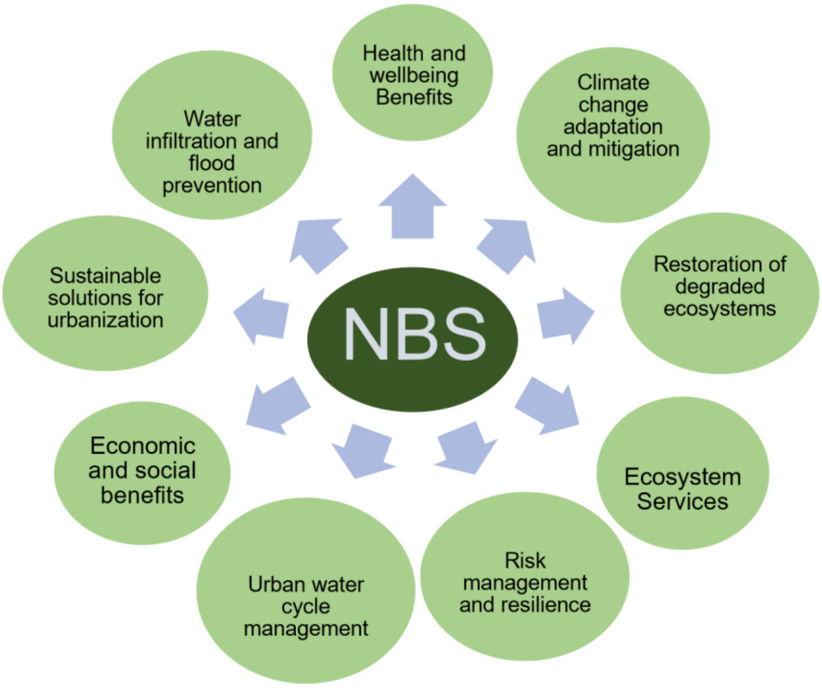


Figure 1: Examples of Nature-Based solutions benefits.

The relationship between water and NBS technologies is addressed in the United Nations World Water Development Report 2018, where is pointed out the use of NBS to achieve a better water quality, to manage water availability, water-related risks, variability and changes (WWDR, 2018). Some of the problems to be solved by the use of NBS are regulation of water supply, regulation of water quality and moderation of extreme events (e.g., flooding) (UNESCO, 2018). Phytoremediation systems for water treatment, besides helping to access a better water quality, can also achieve economic benefits through the price reduction of water treatment plants maintenance, the less energy expended, and the possibility of biomass valorization (Kabisch et al, 2017).

1.2.1 Ecosystem services and functions

The implementation of NBS comes coupled with services that can be generated by the ecosystems, which are mainly categorized into providing, regulating, supporting and habitat, and cultural services. Each ecosystem service can have specific functions depending on the system it refers to. Services that can be provisioned are for example food, water and biomass, while improved air quality, climate regulation and water purification are examples of functions that can be achieved through regulating. In relation to supporting, it’s possible to have habitat support and biodiversity as examples. And finally, in relation to cultural services, these can be for example aesthetic, recreational and educational (Millennium Ecosystem Assessment, 2005).

As mentioned earlier, one of the benefits of implementing NBS is to take advantage of the services and functions generated by nature. In-depth knowledge of the physiology, anatomy and metabolism of plants, including their associations with other living beings such as microorganisms and animals, is essential to create alternatives that benefit human beings and do not cause a major environmental impact.

There are specific ecosystem services that directly influence the quality, availability and management of water. These can be found in the literature as water services, water related ecosystem services, or watershed services. Water services aim to assist the three main challenges for water resources which are: water availability, water quality, and moderating risks and extremes. Some of the water-dependent ecosystem services are based in obtaining products directly from ecosystems such as food, fiber and energy; benefits generated by ecosystem processes such as climate regulation; and supporting and cultural services. These have been classified into three broad groups which are: movement of water (e.g., infiltration and evaporation), the storage of water, and transformation of water (quality) (UNESCO, 2018).

1.2.2 Phytoremediation

The use of nature to restore nature itself has been used for a long time (Shah et al, 2020). Bioremediation consists of the use of organisms, which through metabolic processes remove certain pollutants from contaminated water, air, and soil. This process has proven to be very profitable and its wide use ranges from biological wastewater treatment to solid waste degradation processes. Several advantages including the production of sustainable energy are a consequence of its use (Shah et al, 2020). Nowadays, the use of microorganisms associated with plants roots have been shown very effective (Lakshimi et al, 2017). The need to implement green areas in urban spaces and to create cheap and efficient solutions that can generate economic benefits, has guaranteed its space in the scientific and industrial environment. Phytoremediation come from the combination of two Latin words “plant” and “remedy” (Lakshimi et al, 2017). It consists in the use of plants and their rhizosphere organisms to improve the quality of water, soil and air (UNEP, 2019). The concept is based on the process of self-depuration that occurs naturally in nature, where plants are able to metabolize, transform or remove excess nutrients present in water bodies and soils (Lakshmi et al, 2017).

Although studies exploring phytoremediation as an alternative to remediate contaminated soils are more frequent, there are also those that use this alternative to restore degraded aquatic environments and remediate different types of wastewaters (Fletcher et al, 2020). In the laboratory, the remediation capacity, accumulation and survival of certain plants when exposed to different concentrations of pollutants are analyzed. In addition, some research also looks at the influence of root association and media microbiota on the remediation of wastewater. Regarding the pilot and full-scale systems, these can be CWs, FWIs, vertical flow beds, hydroponic systems, and hybrid systems (Schröder et al, 2007).

The use of phytoremediation applied to wastewater offers advantages but also limitations. It is important to consider the pollutant to be removed and the destination of the water after treatment before choosing the type of system. Often complementary systems are necessary. Phytoremediation is a type of biological treatment that is mainly applied as secondary treatment or tertiary polishing treatment. When applied as a polishing treatment, this technique can remove various micropollutants (e.g. residues from detergent, pesticides, medicines, cosmetics, hydrocarbons) that cannot be removed by conventional wastewater treatment plants (WWTPs) which consist basically of an activated sludge tank and a secondary clarifier (Schröder et al, 2007). According to Ahmad et al (2017), among the advantages of using phytoremediation over other conventional systems is the fact that they are economically attractive options. The maintenance and implementation of these systems require much lower capital investment and are also systems that require little energy during its operation. This fact makes them environmentally friendly alternatives. As reported by Pilon-Smits (2005), $25-50 billion per year is spent for environmental cleanup worldwide. The advantages also include the reduction in the carbon footprint by implementing green areas and the consequent enrichment of biodiversity, the reclamation of wastewater and nutrient recovery (Ahmad et al, 2017). Moreover, it is also possible to generate biomass that can be used in different applications, as how being valorized for energy production. In this case, the biomass can be converted in energy through direct combustion, biogas, and bioethanol production (Pereira et al, 2021).

However, there are some limitations when using phytoremediation in wastewater. Those limitations are related to the pollutants and plants characteristics. Regarding pollutants, these must be bioavailable in the aquatic environment and present where the plants grow in order to be uptake by them. In addition, the toxicity of the pollutant must not jeopardize the plants survival (Pilon-Smits, 2005). Another limitation is the fact that the speed of the remediation process depends on the type and toxicity of the pollutant, as well as on the pollutant uptake process involved: assimilation, degradation, etc (Pilon-Smits, 2005).

There is no legislation directly related to the application of phytoremediation in Portugal. However, as how phytoremediation can be an alternative to remediate wastewater, it is necessary to monitor the toxicological limit allowed for the discharge of the treated wastewater in the environment. There are some documents that describe the importance of implementing NBS, framing the use of these systems in global targets. Among these documents, can be mentioned the

Innovation Policy Agenda Towards Re-naturing Cities and Territorial Resilience (EC, 2015), and the United Nations World Water Development Report of 2018 (WWDR, 2018).

1.3 Plants in wastewater phytoremediation

To phytoremediate wastewaters, the most commonly used plants are aquatic macrophytes. They are constituted, in great majority, of macroscopic plants that actively grow permanently or periodically submerged below, floating on, or up through the water surface of inland freshwater or brackish water bodies (Murphy et al, 2019). Because they are closely related to the metabolism of limnic ecosystems, and since they play such an important role in nutrient cycling and in the recovery of polluted rivers and lakes, these plants become advantageous options for their use in systems such as CWs and FWIs. Besides that, these plants have long and dense roots, which allow the large contact area to create associations with microorganisms, being able to absorb and phytoremediate the pollutants present in water, including metals. Some of the advantages of using aquatic macrophytes for aquatic phytoremediation are: to be able to survive in an environment that receive a high amount of organic load, its roots serve as a site for proliferation of microorganism’s biofilm, possible association with periphytic algae and nitrogen fixing bacteria, grow fast and may produce large amounts of biomass (Eid et al, 2020).

According to Lesiv et al (2020), it is important to note that macrophytes exhibit growth, physiological plasticity and metabolic processes, directly related to changing environmental conditions. This makes them behave differently according to the environment in which they are.

The benefit that the biomass of the plant can generate can be a determining factor when choosing the plant for the system to be implemented. The aesthetic factor is also very important and takes priority in some cases. It is important to mention that in order for them to play an adequate role, the plants should be selected according to the type of pollutant that is intent to be removed and the type of destination that the water will have after treatment. The knowledge about their physiology, anatomy and symbiotic associations are also very important characteristics to take into consideration (Calheiros et al, 2017). Still, choose a native plant is an important decision that can avoid further ecological problems.

Another relevant point is to choose plants with some specific anatomical characteristics such as long and dense root system, high level of degradative enzymes and production of abundant root exudates. There are many plants with the innate ability to remediate pollutants present in the environment. This capacity is directly proportional to the growth rate of the plant, being correlated with the total biomass of the plant (Pilon-Smits, 2005).

Some plants have the ability to hyperaccumulate pollutants. This characteristic gives them a competitive advantage in the phytoremediation scenario. Usually, plants are able to accumulate a contaminant when it is in a biodegraded or bio transformed form. Therefore, these plants have a limit where this capacity is no longer available (Ansari et al, 2020). Hyperaccumulator plants, are able to go to a very high accumulation limit, being able to accumulate more inorganic contaminants, especially metal and metalloids, than other plants (Ansari et al, 2020). In addition, such plants have other advantages over other plants, such as: they can flower in harsh environments, produce greater amounts of biomass, need little maintenance, and have a higher leaf-to-root ratio. Hyperaccumulators have been described for As, Cd, Co, Cu, Mn, Ni, Pb, Se and Zn, where these elements correspond from 0.1 to 1% of the dry weight of these plants (Ansare et al, 2020). However, its use in phytoremediation has some limitations, as how the big amount of waste generated by the consecutives macrophyte pruning's, and that need to be adequately disposed (Ansare et al, 2020).

Through metabolic processes, plants are able to remediate pollutants present in water. Plants used in the phytoremediation process can remediate pollutants through containment/immobilization, mobilization and/or degradation. Their roots have several detox mechanisms and are therefore the ideal entry of nutrients and water into the plant. In this way, the contaminants enter the plant through the roots, where they are absorbed and accumulated. The pollutants can then be stored in the roots themselves, in the stem, or in the leaf, where they are chemically transformed into less toxic contaminants or volatilized in gaseous form (Lakshmi, et al, 2017). Some of these metabolic processes that plants perform to transform, retain or eliminate a pollutant from water are phytoextraction, phytodegradation, rhizofiltration, rhizodegradation, phytostabilization and phytovolatilization. Table 1 summarizes some of these biological mechanisms.

Table 1: Phytoremediation biological mechanisms. Adapted from Lakshmi et al (2017).

Mechanism	Definition
Phytoextraction	The pollutants are absorbed by the plants, precipitated and then translocated to the above ground biomass.
Phytodegradation	Biodegradative mechanism, where organic contaminants are degraded (metabolised), mineralised, assimilated or lignified within the plant cells. The degradation and mineralisation occur through the action of specific enzymes.
Rhizofiltration	Adsorption or absorption of pollutants by aquatic plants either by plant´s roots (rhizofiltration) or by the seedlings (blastofilteration).
Rhizodegradation	Degradation of pollutants by associated microorganisms that consumes the compounds released by the roots.
Phytostabilization	Reduction of the contaminant’s mobility within the vadose zone through accumulation by roots or immobilization within the rhizosphere, thereby reducing off-site contamination.
Phytovolatilization	Contaminants are absorbed by the plant roots which are translocated to the aerial parts and finally to the leaves. Subsequently the pollutants are transformed during the metabolic activities into volatile form in order to be transpired.

The plants play important functions in wastewater treatment, but the individuals who have the biggest role in the decomposition and transformation of pollutants are the microorganisms. Through metabolic processes such respiration and fermentation, microbes can break down organic pollutants into assumed harmless substances (CO_2, N_2, H_2O) (Shahid et al, 2020).

The relationship between plant´s roots and microorganisms are mutualistic. Plants release oxygen and exudates that nourish the microorganisms present in the rhizosphere. On the other hand, the rhizosphere and the root´s microbiota degrades most of the organic compounds present in the region, preventing large concentrations of these pollutants from being absorbed by the plants. Due to this mutualistic relationship, roots are excellent substrates for bacteria to form biofilms (Shahid et al, 2020).

A great diversity of microorganisms can be important in the process of wastewater treatment, from those that have a bioindicator role to those that decompose contaminants. They can be found in the form of biofilms on substrate or in root surfaces (Calheiros et al, 2009c). The biofilm dynamics and the diversity of microorganisms present in water treatment systems such as macrophyte beds, may be influenced by the type of filter/support material (Calheiros et al, 2018), the type of plant (Calheiros et al, 2010), the environmental conditions and the nature of the effluent to be treated (Calheiros et al, 2009a).

The breakdown of different pollutants requires different types of bacteria that works under different environmental conditions. To remove soluble phosphorus in freshwater for example, the presence of a specific type of bacteria called PAO's (Polyphosphate accumulating organisms) is required. They remove phosphorus when they are under oxidative stress and accumulate it in their cells in the form of polyphosphate. For this purpose, it is essential to design a treatment system that transits between an aerobic and anoxic environment (Mina, 2014). For the removal of ammoniacal N, two types of bacteria are essential for the three necessary processes: conversion of ammonia to nitrate (nitrifying bacteria: *Nitrosomonas* and *Nitrobacters*) (NH_4^+ ❼ NO_2^- ❼NO_3^-), and those that carry out the process of denitrification, which is the conversion of nitrate to nitrogen gas (denitrifying bacteria: *Achromobacter, Aerobacter, Alcaligenes, Bacillus, Brevibacterium, Flavobacterium, Lactobacillus, Micrococcus, Proteus, Pseudomonas* and *Spirillum*) (NO_3^- ❼ $N_{2(g)}$) (Mina, 2014). The first processes are oxidative, so an aerobic environment is required unlike denitrification, which is an anaerobic process (Shahid et al, 2020). Regarding sulphate, it is reduced by sulphate-reducing bacteria, which use it as a final electron acceptor in the anaerobic oxidation of organic substrate (Mina, 2014).

In general, autochthonous individuals have the capacity to survive in environments such as wetlands areas, that receive high concentration of organic loads and other pollutants. In contrast, allochthonous individuals, which include pathogenic microorganisms, usually do not survive in the environment offered by plant beds (Mina, 2014). Calheiros et al (2018), points out the importance of rhizo-endophytic bacteria. These pose important roles in the ecosystem and may improve plant resilience and contaminant removal. In addition, they inhabit plant tissues without causing damage to the host plant and have plant growth promoting effects, which help them to cope with various environmental stresses.

The type of microorganisms that can remove pollutants, associated to Floating Wetlands systems, in marine ecosystems was specified by Shahid et al (2020). Beyond bacteria, algae are also part of the main components of the epiphytic microbiota, which colonize the lower surface of FWI. In these systems microorganisms can be categorized into biofilm forming bacteria and water column bacteria, the latter mostly originating from the water around the system (Shahid et al, 2020).

Also, according to Shahid et al (2020), for freshwater environments, actinobacteria was found to be a dominant group, however, Proteobacteria (Mostly actinobacteria) was mainly found in the roots and biofilm samples. Calheiros et al (2020 a), reported that the main groups of bacteria present in the FWI system implemented in a port marine were *Maritimimonas sp*., *Gottschalkia sp*., *Maribacter sp*., and *Lewinella sp* for the water samples, and *Saprospiraceae* and *Gammaproteobacteria* for the plant media biofilm establish in the floating platform.

The choice of plants to be used in phytoremediation systems goes through several criteria as described in section 1.3. However, choosing plants that can add financial feasibility in the place where the project is implemented by plant biomass utilization and element recovery is a differential. Some plants have the capacity to produce end bioproducts that can be used as raw material to produce mostly bioenergy and other manufactures as how craftsmanship and textile products (Edgar et al, 2021).

Lignocellulosic biomass is one of the most promising renewable resources as it can produce biofuel and other value-added compounds. It is composed of 10-25% lignin, 20-40% hemicellulose and 40-60% cellulose. The main feedstock that can be derived from lignocellulose biomass are agricultural residues, virgin wood, forest residues and energy crops such as perennial forage, non-edible seeds and woody forage. The various products that can originate from these feedstocks can be applied in different types of industries (Ning et al, 2021). They can be divided into three categories: i) monosaccharide and sugar alcohol, ii) biodiesel, and finally iii) bio-oil, bio-transport, and syngas (Ning et al, 2021).

Besides the importance of generating energy with low carbon content, plants feedstock can also generate textiles and handicrafts. Some plants with potential for phytoremediation such as *Arundo donax* and *Juncus spp.* have been used in handicrafts in Portugal since past centuries. In the Algarve, cane basketry (*A. donax*) is an activity more characteristic of the riverside areas of the Guadiana Valley. In this technique, the interweaving of the cane makes it possible to make a wide variety of baskets, essential work tools for agricultural tasks and for the storage and transport of products. This technique is still used today, but less frequently (Sousa et al, 2010). Regarding *Juncus spp.,* which was once raw material for making brooms and typical costumes, nowadays is also used in Portugal as vegetable fiber in the art of basketry (Sousa et al, 2010).

Biomass reuse has great significance for the circular economy, as it is a vision of a sustainable society, requiring greater responsibility in the sustainable production of biomass and the recovery of the material value of end-of-life products. Many companies are being charged by consumers to adopt this mode of production, and many others are already applying it (Sherwood, 2020). Besides being part of this concept so important and popular nowadays, the recovery and valorization of biomass through phytoremediation also brings economic advantages through the use or sale of the biomass itself (Jiang et al, 2015).

1.4 Phytoremediation systems: constructed and Floating Wetlands

Constructed Wetlands are engineering systems based on the optimization of the processes that occur in a natural wetland ecosystem in order to eliminate or reduce pollutants from wastewater. These systems have the capacity to treat several types of wastewaters (e.g., domestic and industrial) in different climacteric conditions (Dotro et al, 2017).

The need to produce treated water suitable for a particular purpose requires different treatment goals that go beyond just discharging a mixed treated wastewater stream into a final repository (freshwater or soil). Thus, CWs provide benefits beyond the production of treated water, as how generating the possibility to reuse water, recover nutrients, produce energy and provide ecosystem services (Masi et al, 2018). Besides bringing the possibility of providing these services described above, some particular advantages of CWs are its flexibility in size (Regelsberger et.al, 2020), are easy to handle, are odor-free, can be used for both polishing and secondary wastewater treatment, and it is a cost-effective alternative to conventional systems due to their low typical capital and operating costs compared to conventional systems and negligible energy costs (Calheiros et.al, 2012).

Although many advances have occurred to increase the efficiency of CWs, some limitations still remain, such as the fact that the system needs, in most cases, a much larger space than conventional systems to be built, even if there is flexibility in its size. Its low capacity for nitrogen and phosphorus removal also can be a limitation, although if a mixed system is applied, these nutrients removal levels may increase. Other inconveniences may come from the costs with substrates and previous treatment of residual water, maintenance in case of clogging, and plants management (Dotro et al ,2017).

There are several models of CWs and their distinction, most often, is through the type of macrophyte used and/or the pattern used in the water flow (Dotro et al ,2017). CWs using macrophytes can be classified as: systems with floating aquatic macrophytes and emergent macrophytes. The systems with emergent macrophytes can be classified with respect to its flow, being emergent macrophyte systems with surface flow or with subsurface flow (Vymazal, 2013).

Subsurface Flow CWs are subdivided into horizontal - CWHSF and vertical – CWVSF (Dotro et al, 2017; Kadlec and Wallace, 2008). The systems consist of a filtering base, in which plants are grown, and water percolates through this base, allowing the filtration and depuration of several contaminants present in the sewage (Figure 2). Different physical, chemical, and biological mechanisms are involved in the process, among which are filtration, sedimentation, precipitation, microbial degradation, absorption of nutrients and pollutants by the plants, and the adsorption of nutrients and pollutants in the substrate (Matos and Matos, 2017).

The CWHSF (Figure 2) comprises a substrate on which the emergent macrophytes are growing and the wastewater percolates horizontally below the substrate surface (Dotro et al, 2017).

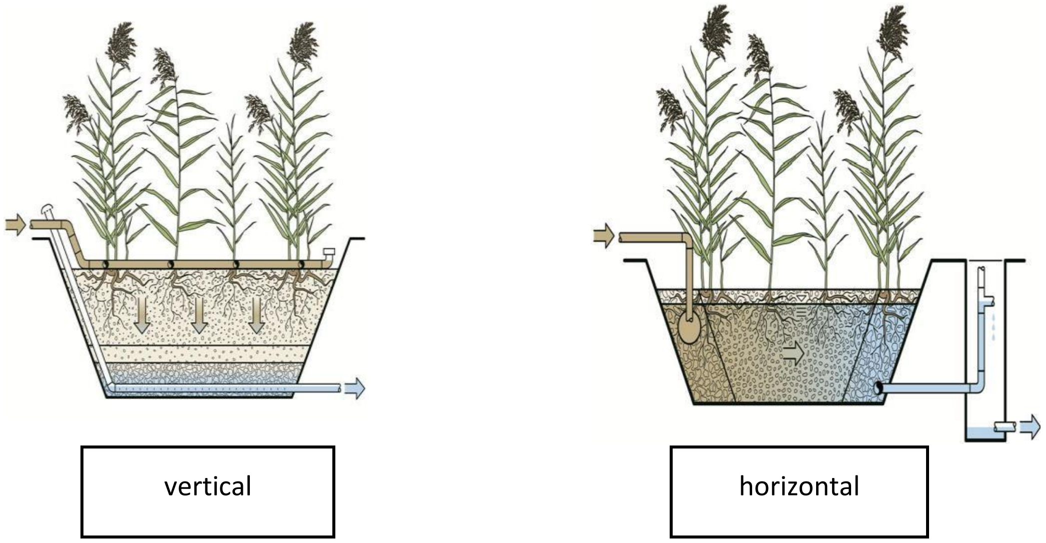


Figure 2: Scheme of subsurface Constructed Wetlands Systems: Subsurface vertical Constructed Wetlands (CWVSF) and Subsurface horizontal Constructed Wetlands (CWHSF). Source: Adapted from Dotro et al, (2017, p.2).

The plants provide oxygen to the microorganisms present in the roots, increasing and stabilizing the hydraulic conductivity (Hill et al,1981). Due to the saturated water condition in CWHSF, mainly anaerobic degradation processes occur. Another important aspect is that an effective primary treatment must be carried out to remove particles and avoid clogging of the filter (Dotro et al, 2017).

CWVSF (Figure 2) consist of systems where the effluent to be treated flows in a vertical way into the substrate being able to follow an ascendent or descendent direction. The wastewater is introduced in intermittent loads onto the substrate by infiltrating through it. Between loads, air re-enters the pores and aerates the filter, allowing aerobic degradation processes to occur. In these cases, it is essential to perform an effective primary treatment to remove particles and avoid clogging the filter (Dotro et al, 2017). Also, according to Dotro et al (2017) Vertical and Horizontal Flow CWs are used in secondary wastewater treatment due to the possibility of filter clogging.

Another type of CW is the so-called French Vertical Flow Built Wetland - FVF. Differently from CWVSF and CWHSF, the French system have been successfully used for raw sewage treatment. This means that treatment is performed at the primary level which translates, among other benefits, into reduced construction costs (Dotro et al, 2017).

The FVF system has two stages (Figure 3). The first stage receives the raw sewage and has three filter cells in parallel, that altern feeding and resting phases (usually 3.5 days of feeding and 7 days of rest). It is this alternation that guarantees the control of biomass growth, maintains aerobic conditions, and allows the mineralization of organic deposits retained in the superficial layer, preventing clogging (Dotro et al, 2017). The second stage is composed of two filter cells acting in parallel, that receive the effluent from the first stage. It provides the polishing of the organic matter and serve as a potentiator for nitrification (Dotro et al, 2017; Molle et al, 2005).

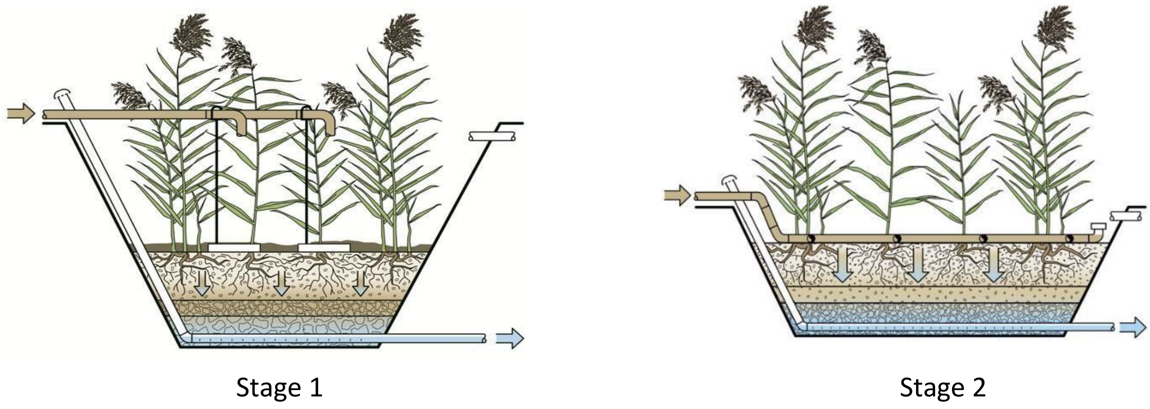


Figure 3: French Vertical Flow constructed wetland systems: Stage 1 and 2. Source: Adapted from Dotro et al (2017, p.2)

As already mentioned, the other classification when considering the type of flow is the Surface flow system. Surface Flow or Free Water Surface CWs (Figure 4) consist of maintaining a water sheet over the soil surface. The presence of microorganisms both in the soil and in the roots of the plants performs the treatment through microbiological action (Salati et al, 2009; Dotro et al, 2017).

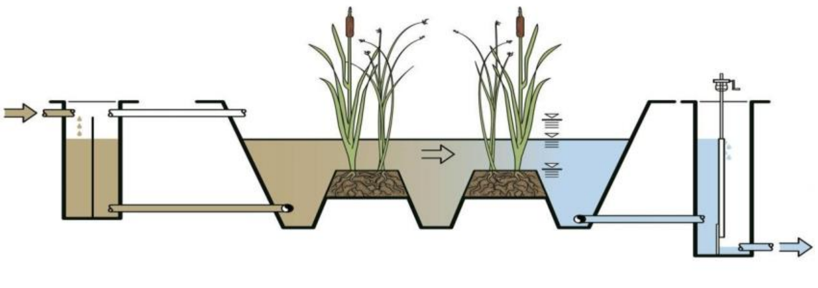


Figure 4:Surface Flow Constructed Wetland System. Source: Adapted from Dotro et al, (2017, p.2)

Surface systems using emergent plants (Figure 4) are developed by means of macrophytes whose reticular system is attached to a substrate, with part of the stem and leaves partially submerged (Bishop and Eighmy, 1989). The surface systems that use floating plants have purifying action due to the absorption of nutrients and metals by the plants, adsorption of particles and the action of microorganisms, as well as oxygen transport through the rhizosphere (Salati et al, 2009).

The Surface Flow CWs can also use submerged aquatic plants (Figure 5), which as the nomenclature explains, are totally submerged, not being exposed to sunlight under the penalty of having their photosynthetically active tissues damaged. Such plants absorb nutrients from well-hydrogenated waters and, therefore, are not always suitable for the treatment of some types of effluents (Bishop and Eighmy, 1989).

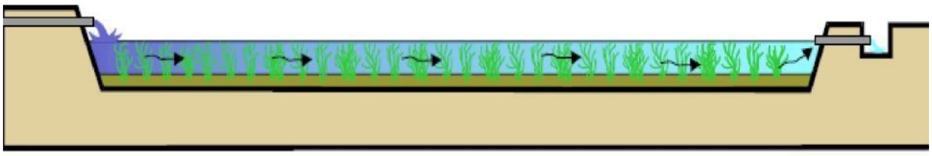


Figure 5: Constructed Wetlands System with submerged macrophytes. Source: Bishop and Eighmy, (1989, p.20).

The mixture of various CWs technologies is known as hybrid CWs. It consists of a configuration generally composed of two stages of various parallel CWs. The most common compositions involve one or more vertical downflow (descendent) CWs - CWVF and one or more CWHF. This arrangement allows the oxidation of ammonia to nitrite and nitrate in the stage with CWVF where aerobic conditions predominate and the reduction of nitrate to nitrogen gas in the horizontal unit where the anoxic/anaerobic condition predominates. There is also Hybrid Multi-stage CWs consisting of more than 3 stages (Rahman et al, 2020; Vymazal, 2013).

Although macrophytes are the main visible organisms active in wetland ecosystems, the incorporation of nutrients by plant biomass alone cannot be considered responsible for the purifying efficiency in these systems. The main role of plants is to provide suitable conditions for the development and action of microbial communities (Matos and Matos, 2017).

The support matrix or substrate used in the CWs, on the other hand, is an extremely important component, especially in subsurface flow systems. It is the support matrix that makes the connection between the components of the system and the main treatment processes. This substrate can be composed of different materials, which each has differentiating characteristics such as granulometric and hydraulic properties, acid-base and surface load properties, mineralogical composition or organic matter content, for example. Such properties will influence the development of the microbial and plant biota supported by the matrix. It is worth noting that some plant species and microorganisms seem to prefer certain substrates to others (Dordio et al, 2013; Matos and Matos, 2020). The criteria for the choice of substrate materials must be determined with respect to their physical and chemical characteristics. Physical characteristics such as porosity, particle size and their distribution and hydraulic conductivity, and the chemical characteristics are determined by the composition of the materials. The properties of the material components of the matrix can determine both the type and strength of the interactions that exist between the substrate and the pollutant molecules on the soil surfaces.

According to Dordio et al (2013) considering that the components of CWs are interdependent, the system design must consider the integration of all parts, thus achieving the necessary synergy for its full operation. Some points regarding the support matrix can be highlighted, such as:

- **The mechanical and chemical resistance of the matrix components**. This characteristic is fundamental since the substrate must be resistant to the operating conditions such as flow and chemical composition of the wastewater, without suffering significant degradation of its properties. There must also be no toxic substances released from the substrate that could contaminate the treated water or biotic components of the system (Dordio et al, 2013; Matos and Matos, 2020).
- **Hydraulic conductivity.** The support matrix needs to allow the inlet flow and the collection of the outlet flow to be evenly distributed. A matrix with low conductivity does not allow for adequate contact of the wastewater with the system greatly reducing its effectiveness (Bassani et al, 2021; Dordio et al, 2013).
- **Porosity**. Porosity is understood as the ratio between the volume of water that passes through the pores and the total volume. There must be space for the treatment (physico-chemical and biological) to take place. Normally porosity of the substate should be between 30% and 45%. A porosity considered adequate also allows for oxygen diffusion, fundamental for the development of plants and microorganisms, hydraulic conductivity, and also allows the CW to achieve one of its objectives, which is the aerobic degradation of carbonaceous organic matter and nitrification (Dordio et al, 2013; Matos and Matos, 2020).
- **Surface area, nutrient availability, and amount of organic matter.** Carbon-rich support materials are able to promote microbiological processes because it can provide the desired amount of carbon for the microbiota metabolism. Porous matrices make it possible for more microorganisms to contribute to the system since they provide a larger surface area for contact and biofilm development (Dordio et al, 2013).
- **Support matrix pH**. It directly influences the nature, development, and activity of the biotic components because different redox gradations, along with the volume of nutrients and environmental aspects, make it possible to create niches where the various biochemical processes occur (Dordio et al, 2013).

According to Yin et al (2017) the most commonly used substrates in CWs are gravel, sand, and expanded clay, but the costs of acquisition and transportation have led to the search for other cheaper alternatives such as solid waste from other activities, as an example bricks. Dordio et al (2013) add that other materials have been used for their sustainable character, such as coconut fibers, cork, and other agricultural wastes and by products. Although cost is a preponderant factor in the choice of material for the support matrix, availability and easy access should also be considered.

As already mentioned, the performance of CWs depends on several factors such as climate, plant species, and granulometry of the filter material. Another relevant factor is the sizing of these systems. Since many variables interfere in the sizing, based on the literature, some considerations can help when thinking on how to dimension a CW. Some methods use hydraulic parameters such as depth, hydraulic distribution rates, detention time and water balance (Oliveira et al, 2020).

Floating Wetland Islands (Floating Treatment Wetlands, Floating Islands, or Floating Wetlands) is a technology that consists in the growth of emergent macrophytes in a floating platform with the roots and associated biofilms hanging in the water column beneath (Calheiros et al, 2020a). This system is applied to the surface of a water body where water receives treatment as it passes through this hanging root-mat. This treatment occurs via biological, chemical and physical processes (Langergraber and Dotro, 2019). The technology intends to mimic the processes that occur in natural wetland systems. The difference is that in Floating Wetland Islands (FWI) plants grow in hydroponic modes instead of being supported in a solid substrate (Calheiros et al, 2020a).

The FWI (Figure 6) is basically composed of plants (aerial part and roots), floating matrix, grown media, microbial biofilm and anchoring system (Shahid et al, 2020).

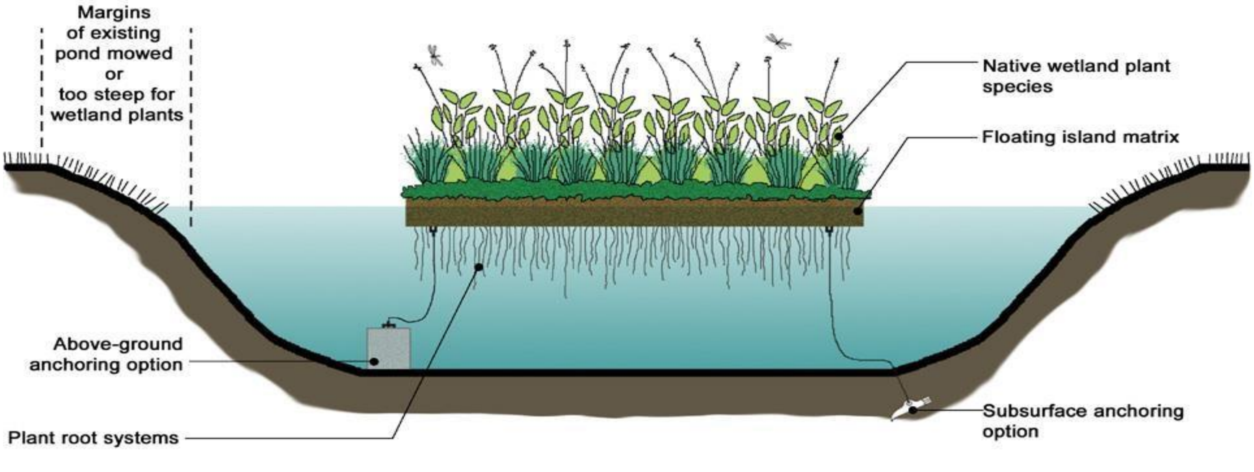


Figure 6: Diagram representing a Floating Wetlands Island system – FWI. Source: Texas A&M (2021)

The floating matrix provides buoyancy to the system and a surface for biofilm formation (Shahid et al, 2020). This surface can be composed of different materials such as plastics, polyester fibers, bamboo, and cork. However, it is currently not recommended to use plastic surfaces due to sustainability issues as such surfaces can release microplastics and toxic substances longevity. Thus, natural floating materials such as bamboo and cork are gaining prominence in current research. (Calheiros et al, 2020a). Another important characteristic to be highlighted, besides the non-toxicity, is the need to be resistant to environmental conditions with respect to water salinity, waving and currents (Shahid et al, 2020). Finally, among the criteria for choosing the matrix it can be also considered the cost and longevity (Calheiros et al, 2020a; Shahid et al, 2020).

In addition to the floating matrix, it may be necessary to use grow media or planting media in order to provide support for plant growth (Shahid et al, 2020). Examples of planting media are coconut fibers, peat, soil, and bamboo (Shahid et al, 2020). As it is a floating system, it is convenient to use an anchoring system, since the islands must be secure enough to prevent them from being excessively displaced by the action of the wind, waves, and currents, and in case the water level rises due to rain, for example (Langergraber and Dotro, 2019). The biological elements of the system are the plants and the bacterial biofilm. Regarding the plants, both the roots and the aerial part are of great importance for the efficiency of the system. Due to the association with microorganisms the roots play an important role in the degradation of the pollutants present in the water. Thus, finer, and more fibrous roots are more efficient in removing total nitrogen, while roots with greater biomass promote greater removal of ammonia nitrogen. In addition, there are characteristics of the roots that influence the type of plant to be selected. The most suitable plants should have long roots and a wide aerenchyma in the rhizomes and root tissues (Shahid et al, 2020). The aerial part of the plants, in addition to contributing to the treatment of water through phytoremediation, also has the role of attracting biodiversity such as, for example, pollinators, birds, among others. In the selection process of the plants to be used, other criteria that should be considered is the preference of native species, that are easily available, non-invasive, perennial, and that can thrive in a hydroponic environment with different salinities (Calheiros et al, 2020a).

Besides promoting water quality, FWIs can provide other ecosystem services such add regulation through capture of atmospheric CO_2, improvement of air quality through particulate filtration/absorption, and the provision of food and biomass from plants that can be used as raw material for composting, ornamental purposes, etc. FWIs can also contribute to biological and genetic diversity by serving as habitat for plants, microorganisms, invertebrates, and vertebrates. They can also generate cultural benefits such as aesthetic, educational, and recreational (Olguín et al, 2017).

Regarding to FWIs biofilms, they play a very important role in removing organic and inorganic pollutants and metals. They can grow both on the floating surface and on the plant's roots. However, research related to the specific species of root bacteria and their role in removing pollutants from water is still underdeveloped. The role of external factors such as temperature and pH on the efficiency of the pollutant removal capacity of specific bacterial communities has been little explored (Shahid et al, 2020). Also, according to Shahid et al

(2020) among the main groups of bacteria that are part of these systems, the endophytic and rhizospheric bacteria play an important role. While most rhizospheric bacteria support the removal of pollutants near the root system, endophytic bacteria mainly act on the pollutants within the roots and stems.

To do the removal of pollutants from the water, the microorganisms that are associated with FWIs perform the following processes: biosynthesis, fixation and biofilm metabolism (Rodrigues et al, 2020). Like the plants that compose the system, the growth of the biofilm also depends on the availability of nutrients and concentrations of compounds that exist in the environment. In this process, both the oxygen and the exudates present in the aerial part of the plant, follow to the rhizome as well as to the reticular surface helping in the formation of a substrate where the colonization of the microbial consortium below the platform takes place (Pavlineri et al, 2017).

The efficiency of the removal process will depend on the metabolism of the biofilm (Pavlineri et al, 2017). Thus, in a FWI, the interaction between plants and microorganisms is essential to achieve good levels of desired pollutant removal. Bacteria promote plant growth, increased biomass production and uptake of toxic pollutants, and alleviate metal toxicity through the production of phytohormones, nitrogenase enzymes, siderophore, metallo-regulatory proteins, and phosphate solubilizing compounds. In return, the plants provide habitat and nutrition for the bacterial communities (Shahid et al, 2020). Some of the advantages of FWIs comprise the reduction of turbulence in the water body, the provision of various ecosystem services, and not needing for implementation a bed with substrate (as in the case of CWs) (Rodrigues et al, 2020).

Some specific factors can affect the performance of FWI's and therefore some design considerations are important. One such factor is the coverage area of the water body containing the FWIs (Lucke et al, 2019). The percentage of cover directly affects the root system of the plants as well as the biofilm growth. Thus, the longer the contact time of the roots with the water column, the better the phytoremediation, pollutant absorption, and metabolic breakdown processes (Lucke et al, 2019). Also, it is more common to implement FWIs in lentic environments such as lakes, ponds, calm stretches of rivers, and dams (Lucke et al, 2019). Furthermore, the oxygen present in the system tends to be consumed by the FWIs while in lotic environments, the rate of passive oxygen transfer will be higher due to diffusion, turbulence caused by winds, and photosynthesis of algae when in the presence of light (Hadley et al, 2008). However, it is possible to deploy in lotic environments as long as there is care regarding anchoring forms, shape and material of the platforms in order to resist in more intense water flows (Lucke et al, 2019; Hadley et al, 2008).

Although anoxic or low oxygen regions are favorable for the process of denitrification by bacteria and the mobilization of metals, in some cases, in order to survive some members of the aquatic fauna such as fish, the oxygen demand of the water can be high. Thus, active reoxygenation of the water column using aerators or air diffusers or passive reoxygenation through free areas for interaction such as the surface and atmosphere has been used to overcome the limitations in oxygen transfer by increasing the efficiency of these processes (Hadley and Tanner, 2008).

1.5 Phytoremediation systems applied to different wastewaters

Phytoremediation systems can be used to treat different types of wastewaters. The following sections will further develop the major types of wastewaters treated by phytoremediation systems: industrial, domestic and urban, agricultural, and aquaculture.

Industrial activities have produced multiple forms of environmental pollution from their production processes. There are several types of industries that produce different types of pollutants. For that reason, each wastewater will have a different composition. Depending on the industrial sector different types of metals, such as Cr, Zn, Pb, and Al, can end up in the wastewater. Several conventional forms of treatment have been used such as membrane filtration, precipitation, nanofiltration, ion exchange, among others, but these processes have significant disadvantages such as high energy use and production of toxic sludge, for example. For that reason, cheaper technologies as CWs or FWIs can be a very interesting option (Saha et al, 2017; Barco and Borin, 2017).

A major challenge that must be overcome by the industrial sector is to make the legal restrictions created to ensure lower environmental impacts compatible with economic and sustainable systems for wastewater treatment. Thus, it becomes essential to conduct technical and economic studies that lead to less costly but effective solutions to comply with the legal dictates. Proper wastewater management is key to solving environmental problems, but also to optimizing the use of resources (Calheiros et al, 2012).

Some types of industries such as textiles and tanneries, use many liters of water in their processes, consequently generating a large volume of effluents contaminated with organic and inorganic pollutants (Nidheesh et al, 2020). However, investing in the installation of traditional effluent’s treatment plants requires a huge investment, becoming economically unfeasible for medium and small sized industries. Thus, industries of this size often end up discharging their effluents directly into water bodies, polluting the water bodies and affecting public health (Nidheesh et al, 2020).

Among the industries that have highly toxic effluents it can be highlight tanneries industries for example. Wastewater that comes from these industries have high loads of organic and inorganic with high biochemical oxygen demand – BOD_5, chemical oxygen demand - COD, suspended solids, nitrogen, salinity and Cr (Calheiros et al, 2012). Other types of contaminants present in some industrial wastewater such as the winery industry, are recalcitrant compounds, such as polyphenols (Nabais et al, 2007; Musee et al 2005; Rodrigues, 2006). Although not appearing in large quantities, phenolic compounds, compounds highly toxic to living beings, can cause environmental damage when released into the environment without proper treatment (Nair et al, 2008).

Among the main sources of contamination and deterioration of natural water environments are effluents from the discharge of domestic wastewater. In mixed or unitary drainage systems that receive water of all types, this type of waste is the most relevant, and its volume is one of the most significant portions of the total flow (Pastor et al, 2008). Although relatively economical and efficient domestic wastewater treatment technologies can be applied for large agglomerations, in the case of small agglomerations, such as rural areas, the use of these solutions is expensive and unfeasible (Pastor et al, 2008). In this scenario, it is necessary to use simpler solutions with lower implantation and maintenance costs. Therefore, the treatment using CWs is one of the most appropriate technologies for wastewater application from small agglomerations. In addition to the low costs involved in the entire treatment process and good efficiency rates, it has an adequate landscape setting, besides being a sustainable technology. Generally, in the rural areas where these small agglomerations are found, there is a large availability of physical space, practically eliminating a possible disadvantage of these systems, which is the need for large areas for their implementation (Pastor et al, 2008).

Metcalf et al (2014) pointed out the typical characterization of untreated domestic wastewater. Regarding this composition, a considerable amount of total solids (537 mg/l low strength concentration, 1612 mg/l high strength concentration), and high organic material concentration (COD: 339 mg/l low strength concentration - 1016 mg/l high strength concentration) were found as being the biggest pollutant concentration. Besides that, nitrites, nitrates, phosphorus (inorganic and organic), chlorides, sulfate, oil and grease, volatile organic compounds, ammoniacal nitrogen and coliform bacteria were part of the composition. Considering the total phosphorus (TP), its average concentration is about 10 mg/l, where 30-50% of the TP comes from sanitary waste, while 50-70% is from phosphate manufacturers in detergents. However, organic phosphorus is not a very important contributor to this type of effluent. Generally, the effluent standard from domestic wastewater discharge can vary from 0.1 to 2.0 m/l. In primary treatment plants the TP concentration of 10 mg/l in the raw effluent is usually reduced to about 9 mg/l while in secondary treatment plants it is reduced to 8 mg/l, this for conventional treatments. Domestic wastewater contains 20 mg/l of organic nitrogen and 15 mg/l of inorganic nitrogen. In treatment systems for this type of wastewater, a limiting factor is oxygen since the heterotrophic organisms eventually consume all the available oxygen in the system (Korkusuz, 2005).

Wastewater of this type contains a high diversity of human pathogenic microorganism's waste that infect the gastrointestinal tract and ends in the domestic wastewater. They are bacteria, protozoa, parasites and virus (Scott et al, 2002). Among the contaminating viruses are: polioviruses, coxsackieviruses, echoviruses, adenoviruses, reoviruses, rotaviruses, hepatitis A virus, and Norwalk-like viruses (Scott et al, 2002; Korkusuz, 2005). Products such as ibuprofen, carbamazepine and clofibric acid, generally at low concentrations (mg/l) are also common in urban wastewater and can be harmful to aquatic systems due to potentially cumulative effects on organisms (Dordio, 2007).

Agribusiness industrial wastewater can be defined as wastewater from the agricultural industry or from family farming activities (Justino et al, 2012). Similarly, aquaculture wastewater is wastewater generated during aquaculture activities (Haung et al, 2019).

Regarding agribusiness industrial wastewater, the most frequent pollutants are high discharges of oxygen-demanding organic compounds, nitrogen, phosphorus, and various organic xenobiotic substances that are applied to soils, crops, or pastures, such as pesticides and pharmaceuticals. Among pollution generated by agricultural activities, livestock farming (e.g., swine farming), food industry (e.g., olive oil production) and the use of pesticides after runoff are challenges still needing to be addressed (Cronk, 1996; Fiorentino et al, 2003; Justino et al, 2012; Matamoros et al, 2012; Dordio and Carvalho,2013). Particularly, the contamination of water by pesticides is still a problem since conventional treatment methods are not prepared for the removal of these pesticides. Several physical, chemical and biological based methods have been tested in order to solve this problem and one of the ways that has proven to be very efficient for treating these types of effluents are CWs (Dordio, 2010) or FWIs (Barco and Borin, 2017). Examples of agroindustry's where phytoremediation systems have been studied and implemented are the wine (Calheiros et al, 2018a), coffee (Sahid et al, 2019) and olive mill wastewater industries (Dordio and Carvalho, 2013).

Aquaculture is also a source of wastewater that is gaining prominence in the pollutant waste scenario, due its capacity to cause serious problems related to the high volumes of water that are discharged containing a high COD, and high levels of phosphorus and nitrogen (Kieu et al, 2021). They may also contain residues from antibiotics used for disease prevention among aquaculture species, bringing another source of environmental problems when disposed into water bodies

(Haung et al, 2019). According to Kieu et al (2021) and Haung et al, (2019) CWs have been used to remove nitrogen and phosphorus compounds from aquaculture effluents because they are efficient, consume little energy, and require low implementation and maintenance costs.

Floating islands with plants are being applied for treat different types of wastewaters. The main applications of this technology in terms of water quality improvement include the treatment of stormwater, sewage, industrial effluent, and water supply reservoirs (Barco et al, 2017). However, the combined use of plants and bacteria for the remediation of polluted river water (Shahid et al, 2019) and polluted sea water (Calheiros et al, 2020 a) in FWIs has been already investigated.

1.6 Aims and outline of the thesis.

The main objective of the present work is to contribute to broader the application of phytoremediation technologies (mainly constructed and Floating Wetlands) for water treatment and management in Portugal, based on available scientific data. Although there are numerous studies on this topic, they are not systematically compiled, and information is not readily accessible for consultation. In this way, the specific objectives of this thesis are:

- Review the existing phytoremediation technologies applied to water treatment and management. This has been carried out through bibliographical research in the main Portugal universities repositories and search tools, to carry out a survey of the works who's thematic is about the use of phytoremediation in the treatment of different effluents in Portugal.
- To identify the main plant species that were most frequently used in phytoremediation systems (CWs and FWIs) in Portugal according to the literature survey.
- To identify the type of wastewater to which CWs and FWIs systems are applied.
- To relate the types of plants with the type of pollutant treated by CWs and FWIs systems as well as the efficiency of the process.
- Identify, for both the full-scale and pilot-scale CWs works researched, the type of system (surface CWs, subsurface, horizontal, vertical, etc.), the level (whether community, industrial, domestic, etc.) and scale of the treatment systems.
- Identify the main ecosystem services provided through CWs and FWIs systems that have been applied at real or pilot scale in Portugal
- To relate plant species used in FWIs systems with the water typology and the type of platform applied to the system.

2. Methodology

For the integrative review, the methodology followed comprised a bibliographic research and field trips to an established CWs and FWIs. It was also implemented under the present study, a pilot FWIs.

2.1 Bibliographic research

Qualitative research was undertaken with adoption of an integrative literature review method, focusing on the theme of phytoremediation in Portugal. This methodological design was chosen because it allows an adequate systematization of knowledge and analysis of results, contributing to the understanding of the subject, based on the analysis of a set of selected publications.

For these literature review, the search of papers, theses and dissertations within the year range from 2000 to 2021, was carried out based on “ScienceDirect” and “Google Scholar” search engines, and Portugal University´s online repositories (Annex 1-Table 18). Also, in the same repositories and together with “Google Scholar” and “ScienceDirect” search engines, a parallel literature review was made of studies published at conferences. The following terms was used: Ecosystem Services AND Serviços de ecossistemas, Constructed Wetlands AND FitoETAR, Macrophytes AND macrófitas, Phytoremediation AND Fitorremediação, Floating Wetlands AND Ilhas Flutuantes, Water Quality AND Qualidade da água.

All the literature was also categorized in a spreadsheet to record the theories, methods, findings of articles read. The categories that were used were: Code, Title, year, macrophytes, System, effluent, source, keywords, authors and country. For the references, the *Endnote* platform was used. Subsequently, a data mapping through graphs to outline the results found was performed.

At the end of the search 214 papers were accounted, where 150 were about CWs, 59 about other relevant theorical bases (e.g., Nature-Based solutions, phytotoxicity.) and 5 for FWIs. The selection criteria were based on the following requirements: papers that describes practical application of FWIs or CWs in the Portugal scenario; papers from other countries that have some more advanced technologies than the ones found in Portugal; relevant theorical base for the technical concepts of CW, residual water, FWI, phytoremediation; and year range between 2000 and 2021.

After reading the 124 selected articles, the collected data were systematized in tables and grouped by type of technology used (CW and FWI). Regarding CWs, after finalizing the table that relate the plant species used and the type of wastewater, it was found that the large number of data generated could hinder the accuracy of the work and its practical applicability. Thus, a new filter was applied to the database using as criteria the five most recurrent plant species, which reduced to sixty the number of articles analyzed involving CWs. On the other hand, for the FWI technology, all five articles surveyed were used to the analysis. From there, new tables were constructed to support the analyses performed. Figure 7 shows a scheme to briefly inform the results obtained regarding the number of works surveyed and the selection criteria that culminated in the framework used.

As a complement to the bibliographic research, a consultation was carried out, by email, with 37 wastewater treatment plants in Portugal that had implemented CWs systems and that were on the list of papers selected for this research. Through this query (Annex 2), it was sought to find out whether the system was still active, with plants developing well and, if so, a report on the physical and chemical analyses of the wastewater entering and leaving the system was asked to be sent. Five treatment plants responded to the query.

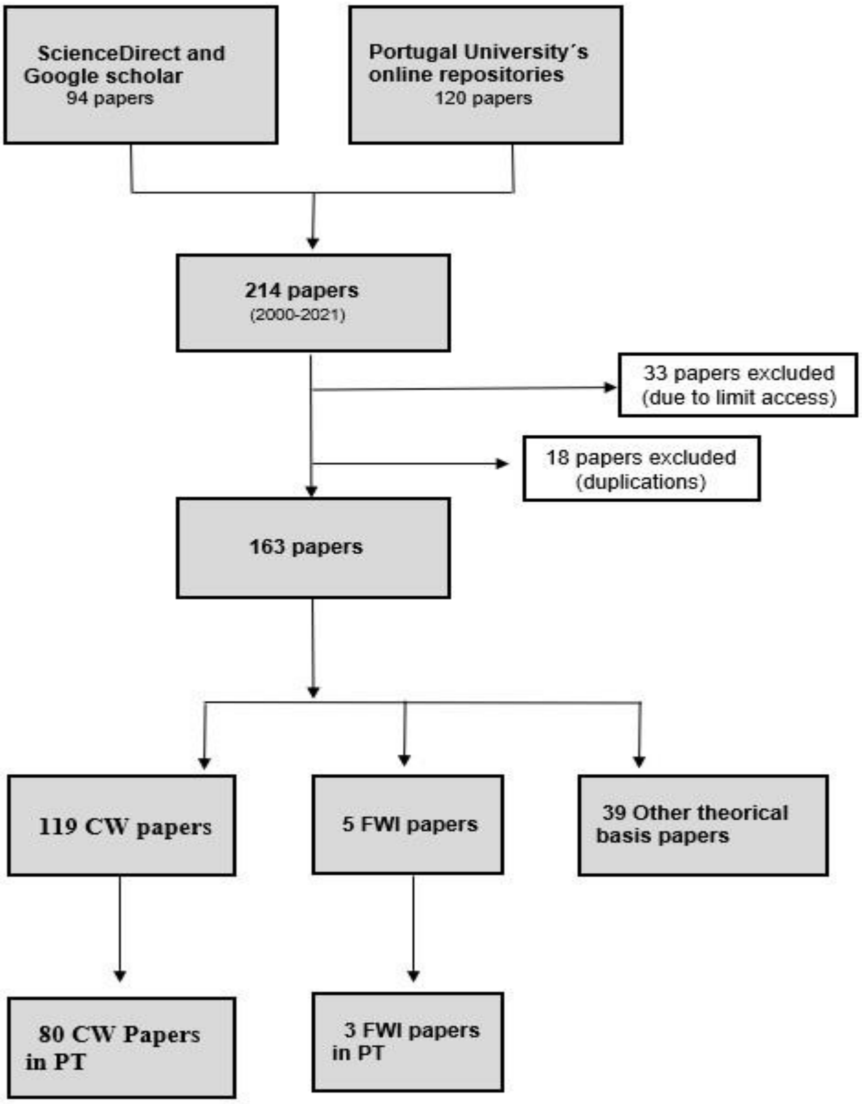


Figure 7: Number of works surveyed and the selection criteria that culminated in the framework used.

2.2 Field trips

To contribute to the bibliographic research, field trips were made to two places of technology implementation in the north region of Portugal: 2 FWIs at a port marina (Porto de Leixões), and a CW at a tourism house, Paço de Calheiros, placed in a rural area.

2.2.1 Constructed wetland at a tourism house

The visit to the real scale CW system that is in operation in the Paço de Calheiros tourism facility was conducted online on 07/08/2021, and had as guide Dr. Cristina Calheiros, who implemented the system in a post-doctoral project in 2010 at Universidade Católica Portuguesa.

Paço de Calheiros is a manor house that is now used for tourism, located in the parish of Calheiros, which is 7 km north of the county of Ponte Lima.

The unit is composed of: a main house, apartments for guests, swimming pool, vineyards, chestnut groves, gardens, orange groves, vegetable gardens and orchards, a small house where the farm machinery is kept and a dinner room. The wastewater coming from the main house and the guest apartments are discharged into a septic tank that already existed and that today performs the preliminary and primary treatment. This transportation occurs without energy expenditure because the terrain is on a slope. Before the CW system project was implemented, the only water treatment that existed was this septic tank followed by an infiltration pit. Therefore, the proposal of the post-doctoral project was to implement a CW unit, that could be a robust, reliable and economically viable system, allowing the reuse of the wastewater after treatment. In this way, the water would be directed to the CW and after that, targeted through a pipe to a pond (Figure 8).

The wastewater that is treated has the characteristics of domestic wastewater (maximum occupancy 40 people and minimum 5 people) but with a very inconsistent flow rate and oscillations in quality and quantity due to the variation in occupancy rate. This means that sometimes the flow is too low, and at other times it is too high. These oscillations are the reason why the system was implemented following a horizontal subsurface sizing, operating in a continuous mode. The subsurface flow ensures that there isn´t bad smell, neither undesirable insect (e.g., mosquitos) (Calheiros et al, 2015).

An interesting innovation implemented by this project was that it was a polyculture with local ornamental plants. The plants used were *Zandeicha aetiopica, Agapanthus africanus, Canna flaccida, Canna indica,* and *Watsonia borbonica.* They were chosen because of their proven efficiency in Constructed Wetlands systems and for having all the characteristics necessary to be suitable plants for this type of system.

The final objectives of this project were: to improve the water quality for potential reuse in irrigation, to study the development of different plant species in the same CW, and to monitor the efficiency of the system when subjected to load variation.

Figure 8: Google earth view of Paço de Calheiros. The arrow indicates where the CW are located.

The final bed therefore had 45 m^2 with a depth of 40 cm. The site chosen for the implementation of the system consisted of an area that had some orange trees around. The process for the CW implementation followed some steps: excavation, opening of waterproofing trenches, implementation of the geotextile waterproofing mesh, implementation of the HDPE geomembrane, welding of the mesh, placement of expanded clay (Leca ® M, Saint-Gobain Weber Portugal, S.A.), finishing with surrounding stones, manhole at the entrance and exit, and implementation of the plants. The plants were transplanted at an approximate rate of 3 feet per m^2. They were collected in the surroundings of the farm.

The finished system consisted of a garden well integrated into the local ecosystem, attracting biodiversity from pollinators to amphibians, where the predominant plant is *C. flaccida* (Figure 9). The maintenance is done usually twice a year by the establishment's own employees who have been trained to be able to perform the process, which is mostly done manually. Studies are being carried out on monitoring the performance and efficiency of the system, the growth of the plants, the quality of the outlet water, as well as the mycorrhizal dynamics, and the microbial part and associated microorganisms. In addition, local biodiversity monitoring was done where pollinators, frogs, insects, earthworms, were observed.

Figure 9: System with flowering plants. Source: Calheiros et al, (2021).

2.2.2 Floating Wetland Island at port marina

The visit to the FWI located in the port marina, Porto de Leixões, took place on 09/30/2020. The first island visited was located near the docks of the Porto Cruise Terminal Marina in Matosinhos (Portugal) (41°10'41.13" N; 8°42'13.99" W) (Figure 10 A).

The system consisted of a set of three interconnected modules. The complete system had 39 pre-cut holes, 37 of which were used for the plants. According to the manufacturer's specifications (Cork Floating Island®, Bluemater, S.A., Porto, Portugal), each one was made of agglomerated cork containing frustoconical holes holding up to 24 plants/m^2 as described in Carecho, (2019). As an anchoring system, the FWI had two individual cables on who's a weight was attached. Each cable passed through a hole in the corner of each side of the FWI and was tied to a metal structure fixed to the top of the wall. This allowed the platform to follow the tide level fluctuations. The plants used were *Sarcocornia perennis*, *Juncus maritimus*, *Phragmites australis*, *Halimione portulacoides*, *Spartina versicolor*, *Spartina maritime*, *Limonium vulgare*. The system was designed to require a minimum of human intervention in terms of maintenance. As this was an experiment, it had a limited duration. It is worth mentioning the pioneering nature of the work in terms of scientifically documenting the application of a FWI in a harbor marina. During the experiment the water in which the floating system was located was characterized both microbiologically and physiochemically.

The second visit (Figurre10 B) was made in the same day. The islands were also located in Porto de Leixões, but in the marina part of Leça (41º 11.0'N; 08º 42.3'W). They were implemented in October 2019 (Campos, 2020). The objective of the work was to investigate the application of FWI as an ecotechnology to promote ecosystem establishment, as well as to evaluate its adaptation in a marine environment. The halophyte plants selected for these pilots were *S. perennis*, *H. portulacoides*, *S. maritima* and *Inula isthmoids*.

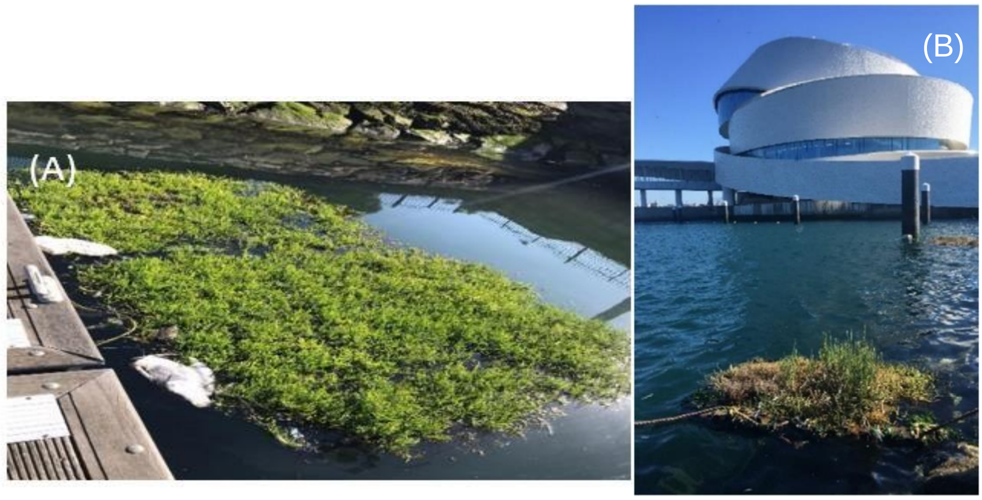


Figure 10: Floating Wetland Island at Marina of Leça (A) and Floating Wetland Island at Marina of the Port Cruise Terminal (B). Source: Calheiros et al (2020a)

The material of the platforms was provided by ECOLINK, Ltda. On site it was possible to observe the mesh (Figure 11 A) and survival of the plant (figure 11B) and the presence of several bivalve mollusks at the base of the FWI (Figure 12A). This association brings a greater weight to the base of the island and may compromise the buoyancy of the platform. In addition, a large biodiversity can also be observed around, mainly associated with plants in the root region and in the substrate, such as fish, crabs, insects, shrimp among others, as can be seen in Figure 12B.

Figure 11: (A) Plant’s survival in the second FWI system visited. (B) FWI Mash

Figure 12: Biodiversity can also be observed around. In the photo, a mussel biofouling can be seen. (A) A shrimp, it was also found around the system. (B).

2.3 Technology implementation: Floating Wetland Island at Porto de Leixões

As an adjunct to the bibliographical research, aiming to observe, in practice, the implementation and maintenance processes of a phytoremediation treatment system using floating islands, it was decided to implement two FWIs. Thus, the systems were set in the port Marina de Leça on June 2, 2021. This marina is integrated in the Porto de Leixões being destined to the anchoring of sailboats and is located in the Atlantic Ocean (41º 11,0'N; 08º 42,3'W).

Each FWI consisted of a module made of agglomerated cork containing 11 frustoconical holes (Cork Floating Island®, Bluemater, S.A., Porto, Portugal). Eight smaller holes (four on each side) were used to place the coconut fiber vases (Vilmorin brand) with the plants (Figure 13 A). The other three larger and centralized holes were used for the anchoring system, which consisted of using a rope that was introduced through one of the holes and passed through the others, with its end fixed to the deck's support base (Figure 13 B). The plants were placed in the pots (Figure 13 C), wrapped in a rock blanket to provide support to keep them upright. The pots had a hole in the bottom that allows the roots to pass through.

The plant used were *S. perennis* (Figure 13 D). One visit per month was scheduled until the beginning of September, that is, three visits. The islands after assembly can be seen in Figure 13 (E).

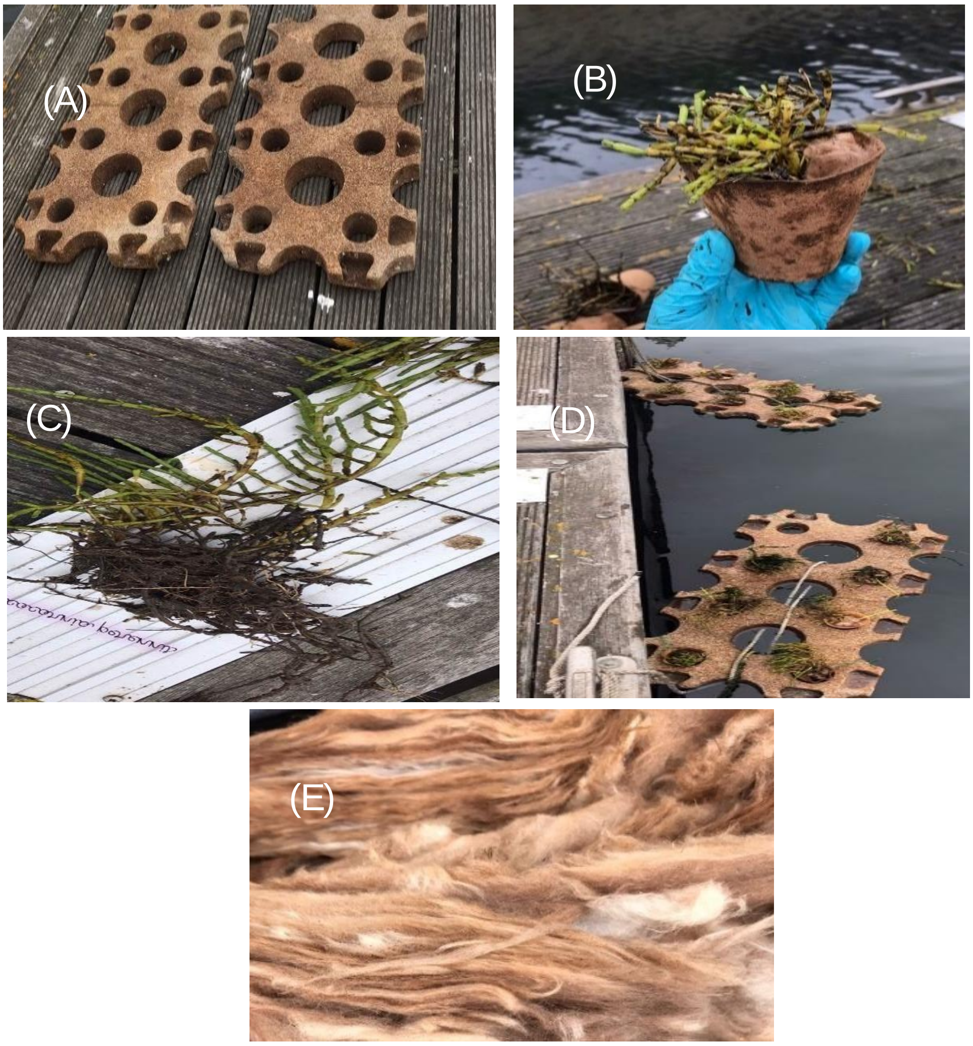


Figure 13: Floating Wetland Island at Porto de Leixões. (A) Module made of agglomerated cork (CORK FLOATING ISLAND®). (B) *Sarcocornia perennis* inside the vase. (C) *Sarcocornia perennis.* (D) System after assembling. (E) Rock blanket.

3. Results and discussion

Among the 83 papers resulting from the bibliographic research selection, approximately 20 papers studied the same CW system in the same location but with different objectives (Table 2). Thus, 41 full-scale and 18 pilot-scale systems were found for CW and two pilot-scale systems for FWI, in Portugal. In order to systematize the data found, initially, the results of the research done for the pilot and laboratory scale systems will be discussed.

Table 2: Papers resulting from the bibliographic research selection

TYPE OF DOCUMENT (N=83)	BIBLIOGRAPHY	SOURCE
Scientific papers (n= 51)		
1	Agnolett et al (2017)	Google Scholar/Science Direct
2	Calheiros et al (2007a)	UCP repository
3	Calheiros et al (2007b)	UCP repository
4	Calheiros et al (2008a)	Google Scholar/ Science Direct
5	Calheiros et al (2008b)	Google Scholar
6	Calheiros et al (2009a)	Google Scholar
7	Calheiros et al (2009c)	Google Scholar
8	Calheiros et al (2009b)	Google Scholar/Science Direct
9	Calheiros et al (2010)	Google Scholar/Science Direct
10	Calheiros et al (2011a)	Google Scholar
11	Calheiros et al (2012a)	Google Scholar
12	Calheiros et al (2012b)	Google Scholar
13	Calheiros et al (2012c)	Google Scholar
14	Calheiros et al (2013a)	UCP repository
15	Calheiros et al (2014a)	Google Scholar/Science Direct
16	Calheiros et al (2015a)	Google Scholar
17	Calheiros et al (2015b)	Google Scholar/Science Direct
18	Calheiros et al (2017a)	Google Scholar
19	Calheiros et al (2017b)	Google Scholar/Science Direct
20	Calheiros et al (2017c)	Google Scholar/Science Direct
21	Calheiros et al (2018)	Google Scholar/Science Direct
22	Calheiros et al (2019a)	Google Scholar/Science Direct
23	Calheiros et al (2019b)	Google Scholar/Science Direct
24	Calheiros et al (2020b)	Google Scholar/Science Direct
25	Calheiros et al (2020a) (**)	Google Scholar
26	Calheiros et al (2021)	Google Scholar
27	Dias et al (2006)	Google Scholar
28	Dordio(2007a)	Google Scholar
29	Dordio(2007b)	Google Scholar
30	Dordio(2007c)	Google Scholar
31	Dordio (2009a)	Google Scholar
32	Dordio (2009b)	Google Scholar

33	Dordio(2010)	Google Scholar
34	Dordio(2011a)	Google Scholar
35	Dordio(2013)	Google Scholar
36	Duarte et al(2010a)	Google Scholar
37	Duarte et al(2010b)	Google Scholar
38	Galvão et al(2019)	Google Scholar
39	Jesus et al(2012)	Google Scholar
40	Jesuset al(2014)	Google Scholar
41	Kabich et al(2017)	Google Scholar
42	Langergraber and Dotro(2019)	Google Scholar
43	Mateus et al (2014)	Google Scholar
44	Mina et al (2011)	Google Scholar
45	Moreira et al (2011)	Google Scholar/Science Direct
46	Nabais et al (2007)	Google Scholar
47	Nogueira et al (2007)	Google Scholar/Science Direct
48	Nogueira et al(2009)	Google Scholar
49	Rodrigues et al (2020)	Google Scholar
50	Silva et al (2015)	Google Scholar
51	Vera et al (2009)	Google Scholar
PhD Thesis (n=8)		
52	Botequilha, J. R. M. (2013).	Lisboa Univercity Repository
53	Carvalho (2008)	Universidade de Coimbra Repository
54	Costa(2014)	Universidade Nova de Lisboa Repository
55	Dordio (2009c)	Universidade de Évora Repository
56	Lino(2014)	Universidade Nova de Lisboa Repository
57	Mendes(2010)	Universidade Técnica de Lisboa Repository
58	Santos (2017)	Universidade Nova de Lisboa Repository
59	Vaz (2016)	Universidade de Aveiros Repository
Master Thesis (n = 15)		
60	Ariscrisnã, P. M. (2012).	Instituto Politécnico de Lisboa Repository
61	Borges, D. R. M. (2015)	Universidade dos Açores Repository
62	Carecho(2019) (**)	
63	Cassoni(2016)	Universidade do Porto Repository

64	Catarino (2016)	Universidade de Coimbra Repository
65	Correia(2014)	Universidade de Évora Repository
66	de Brito (2019)	Universidade do Porto Repository
67	Ferreira(2014)	Universidade do Minho Repository
68	Gonçalves(2009)	Universidade de Évora Repository
69	Mavioso (2010)	Docplayer.com Repository
70	Oliveira (2008)	Universidade de Coimbra Repository
71	Pinto (2009)	Universidade de Coimbra Repository
72	Rio (2017)	Universidade de Açores Repository
73	Ribeiro |(2016)	Universidade do Porto Repository
74	Seco(2008)	Docplayer Repository
75	Silva (2016)	Google Scholar/Science Direct
Conference Abstract/proceedings (n = 6)		
76	Calheiros et al (2003)	Google Scholar
77	Calheiros et al (2011b)	Universidade Católica Portuguesa Repository
78	Calheiros et al (2013b)	Universidade Católica Portuguesa Repository
79	Machado et ao(2006)	Universidade do Minho Repository
80	Mina et al(2009)	Universidade Católica Portuguesa Repository
81	Quadros(2019)	Universidade de Açores Repository
Repport (n = 3)		
81	Duarte et al(2010b)	Google Scholar/Science Direct
82	Korkusuz (2005)	Google Scholar/Science Direct
83	Campos (2020) (**)	Universidade do Porto Repository
(**) FWI Studies		

3.1 Constructed and Floating Wetlands pilot systems

Table 3 correlates the main characteristics of the pilot scales CWs that were found in the literature. It is possible to conclude that the most used CWs type was the horizontal subsurface flow CWs, with only one paper mentioning the vertical subsurface flow CWs. No research using the surface CWs system was found. In addition, the systems were mainly used to perform secondary and tertiary treatment. Other finding is that domestic wastewater was the target wastewater in the largest number of papers, followed by other types of wastewaters (mostly synthetic) and industrial wastewater respectively. In the case of industrial wastewater, the pilot systems were applied to tannery wastewater and winery wastewater.

Table 3: Constructed wetland pilot scale systems, operating with subsurface flow mode, for different typologies of wastewater and treatment stage in Portugal

References(*)	CW dimensioning		Wastewater typology					
	Sub superficial flow		2º treatment	3º treatment	Industrial	Agricultural	Domestic	Other
	SSV	SSH						
24, 37, 36		1	1		1			
26		1	1		1			
204		1		1			1	
47		1		1			1	
72		1		1				1
83		1	1			1		
77		1		1				1
103		1		1			1	
80		1		1			1	
79		1		1			1	
192		1						
133		1	1				1	
132		1	1				1	
203		1	1					1
110		1						1
23		1	1		1			
57	1		1					1
137		1	1				1	
TOTAL 18	1	17	9	7	3	1	8	5

(*) The numbers represent the numbering of bibliographic references.
SSV= subsurface vertical flow; SSH = subsurface horizontal flow

Regarding the pilot systems found for FWIs, only two systems applied in Portugal were found, both intended for application in port marine.

3.2 Constructed and Floating Wetlands real scale systems

It was found in the literature review 41 real scale CWs systems in Portugal. Given the extension of the list of the real scale systems can be found in Annex 3 (Table 19) with the details. It is possible that most of the systems were carried out at community scale, being 2 systems at industrial scale and 2 systems at residential scale.

In relation to CWs, according to the data in Annex 3 (Table 19), it is observed that there is a predominance of the use of CW in the second stage of treatment (90%). As for the type of water treated, a predominance of 85% was observed in the treatment of domestic wastewater. Also, all the systems found consisted of CWSH. In the 15 systems that contained information about the type of substrate used, 3 used expanded clay, 6 sand, and 6 gravel. Main CW real scale systems (65%) are found in the center of Portugal and concerning to FWI they were no records of real scale systems implemented.

3.3 Constructed and Floating Wetlands plants

3.3.1 Constructed Wetlands plants

The plant species found in the different papers that referred to “Constructed Wetlands” as a tool to treat various types of wastewaters are shown in Figure 14. They refer to the pilot systems and real scale systems. The five most used plants were the species *P. australis, T. latifolia, A. donax, I. pseudacorus* and *C. indica*. However, when referring to the plants that were applied in the full-scale systems, the species *T. latifolia, P. australis,* and *Juncus spp.* were the most used, and regarding the pilot-scale system the species *T. latifolia, P. australis,* and *Juncus spp.* were the most used.

According Seco (2008) it is important to highlight some relevant aspects for each species. Although not considered an ornamental plant, *P. australis*, commonly known as reed, is a native plant in Portugal; even though this plant generates a flower not very attractive, they can attract pollinators to the system, mainly insects. They have an internal aeration system through the intracellular spaces, and the formation of 'protective' cellular layers near the surface of the underground organs. This plant had great prominence when related to CWs systems in Portugal and much is due to its physiological adaptations, anatomical characteristics and the fact that it is a cosmopolitan specie). It is also a very easily species to find in natural environments in Portugal. It can also be found in places with low oxygen concentrations and high organic matter content, as configured in some types of wastewaters (Seco, 2008). In addition, their roots are long enough to reach the pollutants, and the plant has a rapid growth and reproduction rate (Seco, 2008). Some studies also indicate that the roots of *P. australis* harbor more diverse and abundant bacteria colonies in its biofilm when compared to other plants, as shown for example in the study by Vymazal et al (2001) when comparing *P. australis* and *Phalaries arundinacea*. However, when analyzing the study done by Calheiros et al (2009) they did not find much difference in amount and diversity of bacterial colonies when comparing *P. australis* and *T. latifolia* treating an industrial wastewater (tannery wastewater).

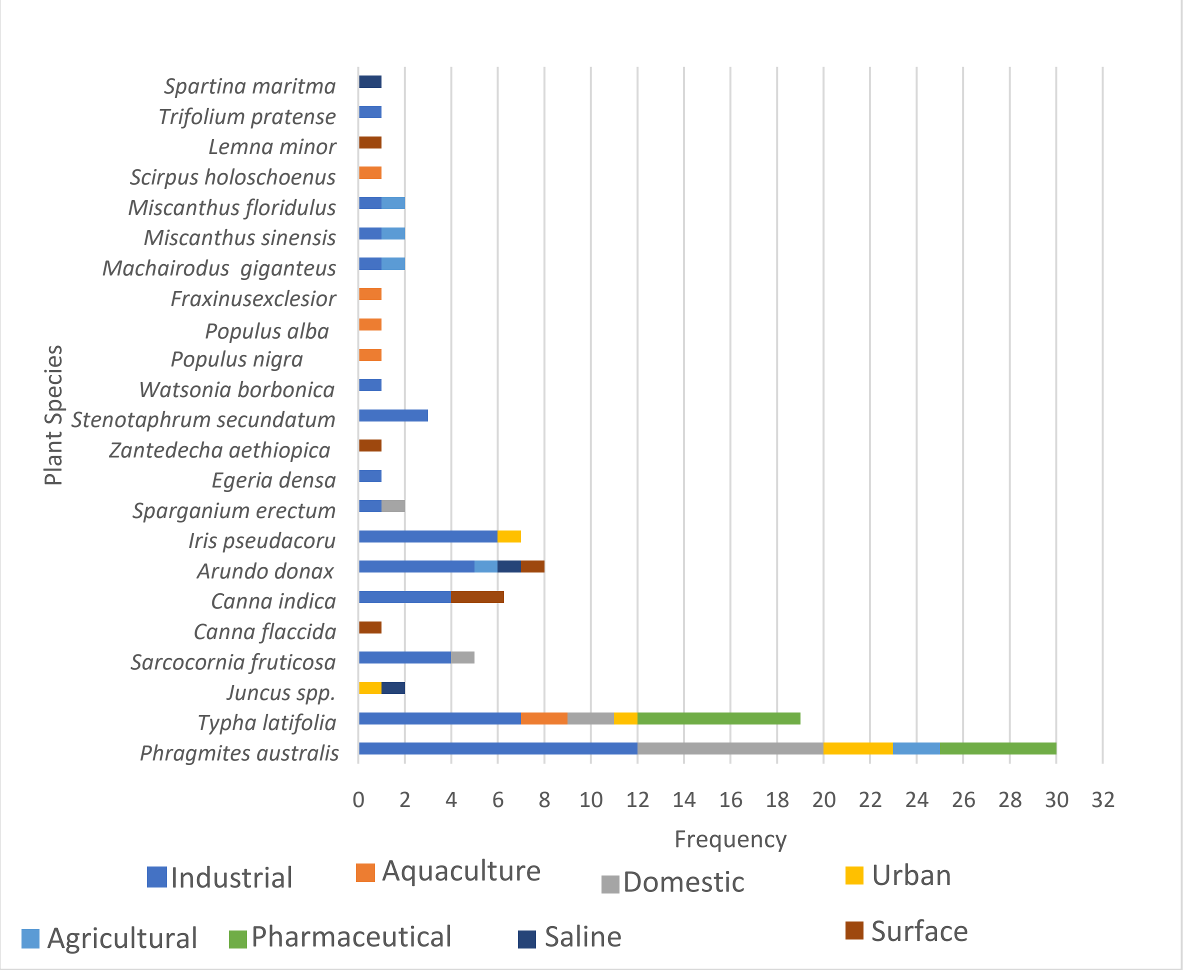


Figure 14: Plant species found in the different papers that referred to "Constructed Wetlands" as a tool to treat various types of wastewaters.

T. latifolia was the second most used plant in CW systems according to the literature survey. It is also a native plant in Portugal, growing in ditches and beds of lentic streams with soils soaked in fresh water for most of the year (Wießner et al, 2002). They have roots long enough to remove pollutants from CW systems, developing underground rhizomes that allow them to spread and form large populations on the banks of ponds, streams, marshes, estuaries, etc. It is a plant of rapid growth and dissemination and can tolerate various types of temperature and soil. These characteristics make its use in CWs an advantage, as it can be used to treat different types of effluents (Wießner et al, 2002). Like *P. australis*, *T. latifolia* do not have a very attractive inflorescence. They appear in late summer and in some places their female flowers are used to ward off insects. Regarding the physiological processes, *T. latifolia* is able to release a considerable amount of oxygen to its rhizosphere, which contributes to its efficiency in the removal of certain pollutants, especially those that are removed by aerobic bacteria, as highlighted by Wießner et al (2002).

Arundo donax, or reed cane, was the third most found plant in the literature search. This species has a more developed root when compared to other species, and because of this, as stated in relation to *P. australis*, a greater diversity and abundance of bacteria can be found in its rhizosphere (Costa, 2014). Calheiros et al (2010) when comparing the bacteria found in the rhizosphere of *A. donax* and *S*. *perennis*, found out that there was a greater abundance in the rhizosphere of *A. donax.* Also, the resilience of this species can be observed in several studies, such as in Calheiros et al (2014b), which show its ability to survive in very adverse conditions such as salt stress and different temperatures. As much as it shows these characteristics that contribute to its choice in phytoremediation systems in Portugal, this plant is invasive in the country (listed in Decree-Law No. 92/2019, July 10) having as preferable environments for invasion water lines, dikes, wetlands, marshes and coastal marshes. It is also very common on the edge of roadsides and agricultural areas. Today it is growing all over the country, except at high elevations. In addition, they have a very fast growth, easy distribution, and their flowering happens from August to October, being not very attractive (Costa, 2014).

For the purposes of this work, ornamental plants are considered those that have a very attractive look, with imposing, colorful and showy inflorescences, and the species *I. pseudacorus* and *C. indica* were the most commonly ornamental plants found in Portuguese CW systems. Besides them, other ornamental species are also studied as shown in Figure 15. The choice of these species, besides their aesthetic advantage, also brings several ecosystem services as mentioned in Section 1.3. It is important to highlight that after being used with the purpose of phytoremediation in highly contaminated sites, it is advisable to perform toxicological studies in order to assure that there is no risk for humans if they use the flowers for inhouse decorative purposes.

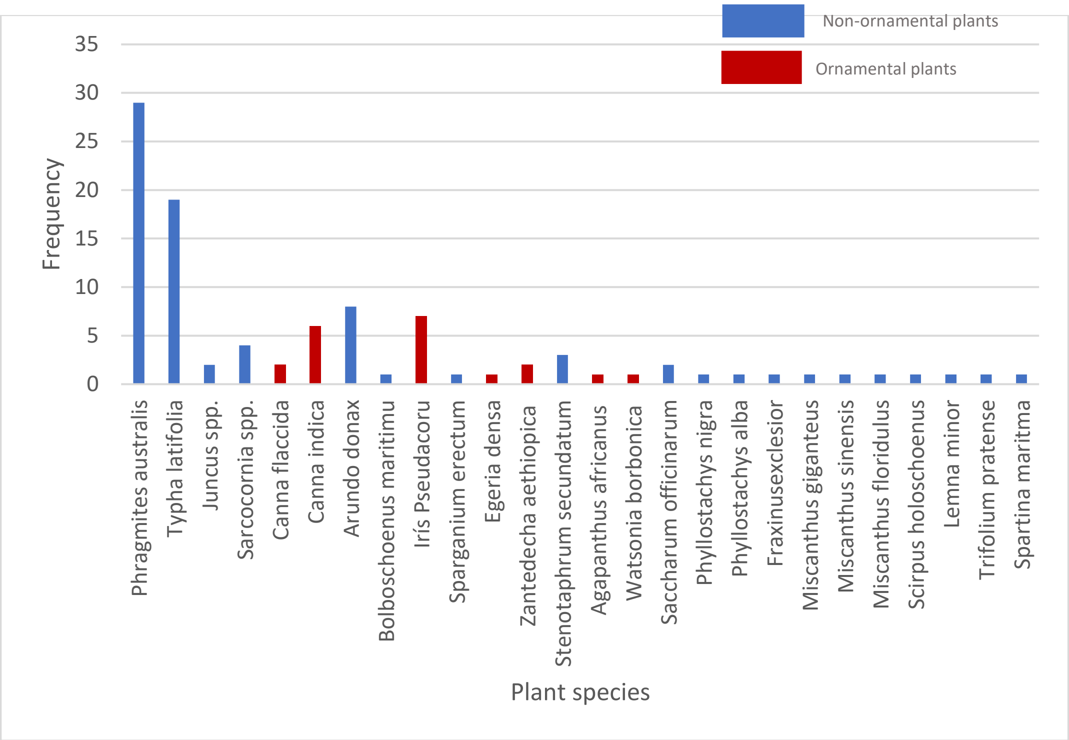


Figure 15: Comparison between the frequency of ornamental species and some other non-ornamental species cited in the papers found in the bibliographic research. The red bars indicate the ornamental species.

Canna indica is a plant from South America, Central and Southeast USA that was introduced in Europe and today is already naturalized in the European territory. They can be found mainly in places where the climate is temperate-warm during the summer. This plant produces an imposing flower of various colors such as yellow, red or bicolor and blooms in spring and summer, remaining until mid-autumn (Flecher et al, 2020)

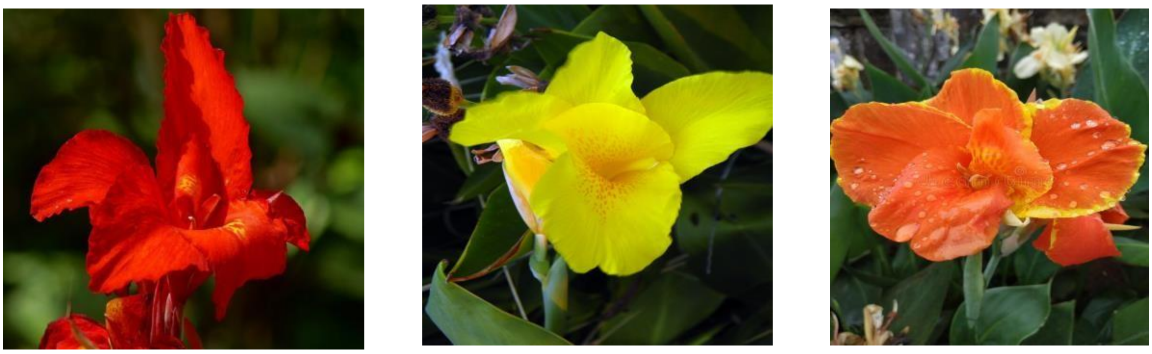


Figure 16: *Canna indica* flowers..Source: Flora-on.pt

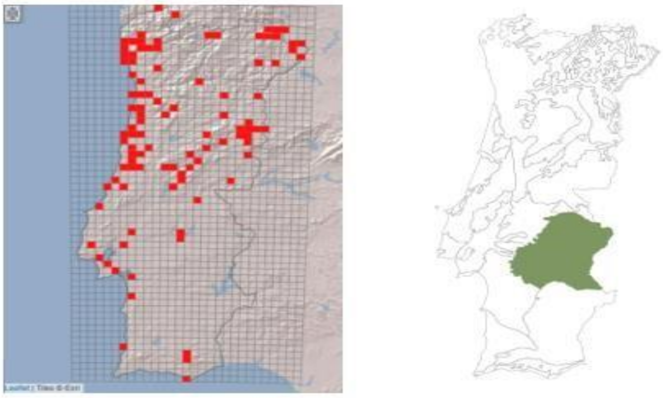
Because it is a plant with such an attractive flower that does not need high maintenance, this species has been widely used in Portugal for ornamental, landscaping and decorative purposes. It is a plant that multiplies easily through rhizome divisions and has a large root area and biomass (roots between 18-20 cm), being long enough to reach major pollutants when used for phytoremediation purposes (Flecher et al, 2020). In a study that evaluated the arbuscular mycorrhizal fungi (AMF) inhabiting *C. indica,* when treating domestic wastewater through Constructed Wetlands systems, Calheiros et al (2019c) showed that this plant was the species with the highest richness and diversity when compared to the other plants *(C. flaccida* and *W. borbonica).* Regarding phylogenetic affiliation of AMF colonizing roots of *C. indica*, the main species found were *Acaulospora sp.* and *Glomus sp.*

The species *I. pseudacorus*, or yellow lily, is a rhizomatous and cosmopolitan plant that generates, in mid-April/June, an imposing yellow flower (Figure 17). It is a plant with wide distribution in Portugal and has been receiving prominence in the field of phytoremediation because, in addition to its beauty, it can survive at variable conditions and does not require special care besides than constant humidity (Seco, 2008). Calheiros et al (2018) conducted a study that pointed out the bacteria associated with the rhizosphere of *I. pseudacorus* in a CW mesocosm receiving winery wastewater. This research obtained a positive result using a cork substrate, in which the plant rhizosphere and the biofilm associated with the shoots could show a good adaptation. Among the bacteria found in the root, the genus *Rahnella* was the most abundant, followed by *Pseudomonas* and *Pantoea*. In shoots, most bacterial endophytes belonged to the genus *Pseudomonas*, while the remaining isolates were affiliated to 4 other genera: *Rhizobium, Flavobacterium, Duganella* and *Xanthomonas*.

Figure 17: *Íris pseudacorus* Flowers. Source: Flora-on.pt

Figure 18 shows the distribution of each plant mentioned in the Portuguese territory. The figures referring to *T. latifolia*, *A. donax*, P*. australis* and *I. pseudacorus* were found in the data platform flora-on.pt, while *C. indica* was not listed in the database of this website and had to be found in another platform. This is probably due to the fact that this plant is limited to a specific region of Portugal (northern southeast), which curiously did not restrict the consideration of its use for phytoremediation systems. Regarding the other plants, it was noticed that the most abundant and well distributed species is *A. donax*, probably due to its easy reproduction, adaptation and distribution, which made it a successful invasive species in the country. In any case, this species clusters closer to the coast, as does *P. australis*, which is the next most abundant plant. This will help explain in future topics its ability to tolerate saline wastewater and even the choice of this species for FWI systems in a marine ecosystem. *P. australis* is more concentrated in the central coastal region, tending towards the south of the country, where the climate is warmer. *I. pseudacorus* was the plant more evenly distributed throughout the Portuguese territory, although it shows a preference for the north coast, where temperatures are milder. *T. latifolia* was the second less abundant plant according to the maps found, concentrating itself a little more distributed in the central/northern region of the country, having a slight preference for the coast.

Arundo donax *Iris pseudacorus* *Praghmites australlis*



T. latifolia *Canna indica*

Figure 18: Distribution of selected plant species mentioned in the Portuguese territory: *Typha latifolia*, *Arundo donax*, *Phragmites autralis* and *Iris pseudacorus*. Source: Flora-on.pt e jb.utad.pt (*C. indica*)

3.3.2 Floating Wetlands islands plants

The plants found in the two FWI systems in the literature are shown in Figure 19, and its distribution in the portuguese terrirory in Figure 20. It is possible to see that the plants that were commonly used in both studies were *S. perennis*, *H. portulacoides*, and *S. maritima*. Due to the reduced number of studies found for this technology, it is necessary to point out that the other plants, although not used in both studies, have equal potential to be explored in this type of study. However, only the characteristics of the Top 3 previously described will be discussed below. It is important to note that the works consisted of a FWI located in port areas on the coast, implying a similar type of wastewater characterized by being salt water and with emerging pollutants such as hydrocarbons. This fact influences the choice of plants and their efficiency and therefore, these should not be considered as a representative average for the best plants to be used in FWI in Portugal overall. According to the review undertaken by Pradhanang et al (2018), other common plants to be used in FWI are *Carex stricta*, *Pontederia cordata*, *Hibiscus moscheutus*, and *Spartina pectinata*.

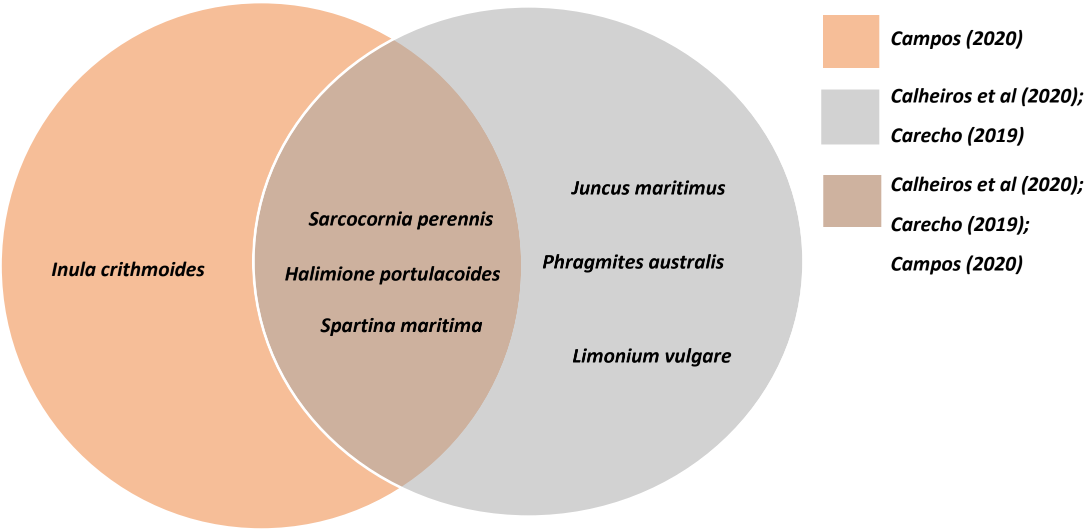


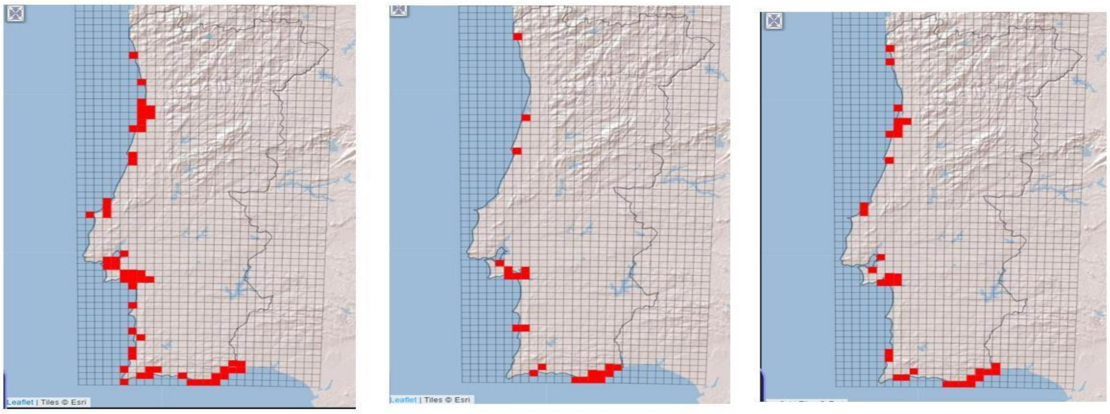
Figure 19: Plants used in the Floating Wetlands systems studies.

Sarcocornia perennis was the plant with the highest survival rate found by both systems, besides being the most dominant in the study done by Campos (2020), and the one that achieved the highest survival rate in the experiment done by Carecho (2019). It is a halophytic plant, present in continental Portugal, being native to the region (Carecho, 2019). It is characterized as a woody perennial subshrub of marshes, especially in bare or sparsely vegetated areas on firm and muddy sand, and gravel. Its location in the country is restricted to the Portuguese coast, relatively well distributed, with a slight preference for the southern region, where the climate is warmer (Pereira, 2012). The Shannon diversity index found in the study by Calheiros et al (2020), was higher for *S. perennis* than for the other species, which may have an influence on the ability to remove pollutants from wastewater. Also, according to this study, Chloroflex i, Cyanobacteria, and bacteroidetes were the main phyla found in the rhizosphere of this plant. In addition, Ventura and Sagi (2013) also cites that *S. perennis* is rich in essential fatty acids, minerals, and antioxidant compounds such as polyphenols, besides being highly tolerant to salinity variations. This plant had a positive result in both works analyzed. However, Carecho (2019) concluded that the dose of 1550 mg/L of TPH (petroleum hydrocarbons) could be lethal for the plant, besides the fact that this plant did not influence the removal of this pollutant from the water tested in lab scale experiments.

Halimione portulacoides is a species present in Portuguese territory, notably in mainland Portugal, being halophytic and native to the region (Aleixo, 2013). Duarte et al (2007) cited the capacity of *H. portulacoides* to absorb Cd and Ni, when with citric acid in phytoremediation processes. This characteristic gives the plant an advantage when used to phytoremediate wastewater contaminated with these metals. In addition, the plant also has a high biomass, making it possible to reach high metal content in its roots and other aboveground organs (Aleixo, 2013). However, the plant did not survive till the end of the FWI experiment by Carecho (2019), only in the pilot system implemented by Campos (2020) where, although it survived, it did not show great prominence in its development. Regarding the microbial association in the rhizosphere, Calheiros et al (2020) had as a result a much lower Shannon index of *H. portulacoides* when compared to the species *Sarcocornia perennis*.

The last plant species related to FWI systems to be described is *S. maritima*. This plant naturally inhabits sea sands. It is considered a native plant of Portugal and is distributed along the coast of the country and in the Azores archipelago. It is perennial and grows from a creeping rootstock (Carecho, 2020). It is described as having a high capacity to tolerate saline environments

Carecho (2019) and Campos (2020) also point out a good adaptation of this plant to saline environments, although not having great prominence when compared to others used for the same purpose. This plant presented 67% survival in the FWI experiment done by Carecho, (2019). Regarding the rhizosphere, the main phyla of prokaryotes found were proteobacteria and the Bacteroidetes.



Halimione portulacoides *Spartina maritima* *Sarcocornia perennis*

Figure 20: Distribution of plant species in Portugal: *Halimione portucaloides*, *Spartina maritma*, Sarcocornia perenis. Source: Flora-on.pt

3.4 Wastewater typology associated to constructed and Floating Wetlands vegetation

3.4.1 Constructed Wetlands wastewater treatment

Figure 21 shows the 5 most used plants among the surveyed works related to the type of wastewater to which they were submitted when applied in CWs. The typologies found were industrial wastewater, wastewater from aquaculture systems, domestic wastewater, urban wastewater, agricultural wastewater, water contaminated with pharmaceuticals, and water with high salt concentration.

Based on the literature, the main Portuguese industry to apply CWs for wastewater treatment was the tannery industry. Of the surveyed papers treating industrial wastewater, 90% used wastewater from tanneries. Moreover, two papers were also found that treated wastewater from the wine industry. Furthermore, plants that have the ability to remove pharmaceuticals from wastewater can be considered potential candidates for treating wastewater from the pharmaceutical industry and domestic effluents.

It is important to note that wastewater from industries has a composition that varies according to its production process, which implies the use of different plants for different industrial sectors.

According to Figure 21, the plants that were most used to phytoremediate industrial wastewater were respectively *P. australis* with 12 different implemented systems, *T. latifolia* with 7, followed by *I. pseudacorus* with 6*, A. donax* with 5, and *C. indica* with 4. These species were basically used in the phytoremediation of tannery wastewater. Among the top 5, the only plants that were used to treat another typology of industrial wastewater were *A. donax*, which received effluent contaminated with metals and *I. pseudacorus*, which was present in the first system studied (Calheiros et al, 2018) and which treated winery wastewater. The winery treatment system consisted of CW mesocosms placed after a winery with a production of 6000 bottles per year, on a farm located in northern Portugal. Although the focus of the work was to analyze the bacterial communities associated with the rhizosphere and plant tissues, a satisfactory result was obtained when this plant was associated with a substrate made of cork (analysis of the influence of the substrates found will be discussed in the next topic). In addition, effluents from wineries have high numbers of TSS and COD.

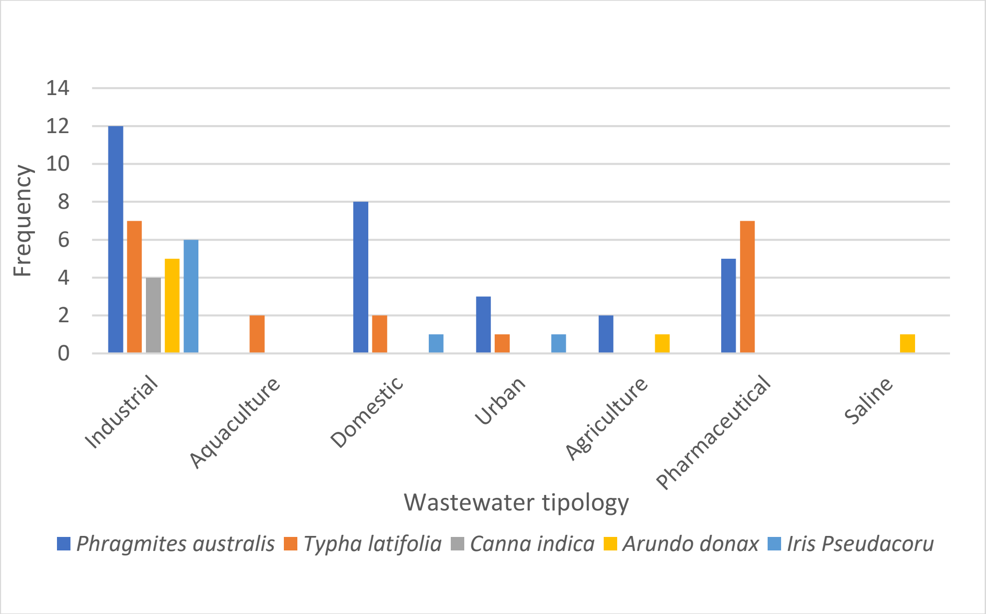


Figure 21: Top 5 plants versus wastewater types.

Table 4 compares the removal efficiency of each of the top 6 plants by averaging the wastewater values before and after passing through the CW system found in the analyzed papers. One can observe the ability of *I. pseudacorus* in decreasing these wastewater values. Related to the microbial communities, the high acidity of the effluent does not seem to have affected the ability of the rhizosphere to remove the necessary pollutants as much. The groups of bacteria that survived these conditions in the rhizosphere were mainly of the *Rahnella* group, and *Pseudomonas* (Calheiros et al, 2018).

The second system treating winery wastewater was presented by Silva (2015) through a biodegradability study using CW experimental units. The plants used were *Sparganium erectus* and *Egeria densa*. In this work the treatment efficiency of effluent was compared using intensive aerobic biological processes, anaerobic biological process and extensive biological processes using CW; it was proven the highest removal efficiency of pollutants with loads up to 5000 mg/l. TSS, COD and pH improvement was achieved by Silva (2015). The systems that had the best efficiency were those using LECA as support matrix and *S. erectum*.

Regarding metals phytoremediation capacity, a laboratory scale study evaluated the efficiency of *A. donax* in the removal of Zn and Cu from wastewater (Costa, 2014). In this experiment where pots containing soil were used, a greater accumulation of Zn was obtained in the rhizomes and leaves and a greater accumulation of Cu in the leaves and roots. The soil-plant system was able to retain above 90% of the affluent Zn/Cu, resulting in wastewater scrubbing. Furthermore, the removal percentages by the plant were usually higher than 8% and 3% for Zn and Cu, respectively. These results show a potential capability of using *A. donax* in CW treating wastewater from metal-rich industrial effluents, such as metallurgical plants.

Calheiros et al (2014a) made a review about the use of CWs to treat tannery wastewater in Portugal. In this publication it was pointed out that *T. latifolia*, *P. australis* and *A. donax* had good results in depurating chromium rich wastewater in terms of survival and propagation. That's mainly because of its capacity to survive receiving a high saline wastewater and to phytoremediate metals. In contrast, *C. indica* and *I. pseudacorus* have not proven efficient in being used for such purpose, as they did not thrive in such wastewater (Calheiros et al, 2006). Confirming what was stated by Calheiros et al (2014), table 4 shows that *P. australis* and *T. latifolia* indeed manage to remove Cr from wastewater unlike what is shown for *I. pseudacorus*. It can also be observed in this table the ability of *P. autralis* and *T. latifolia* to reduce the levels of COD, BOD_5 and SS which are factors present in large concentrations in wastewater from the tanning industry.

Table 4: Removal efficiency of each of the top 5 plants used in Constructed Wetlands. The abbreviation n.a means not available

Parameters		**Plant Specie**					**References/nº**
		(Input: Cmin - Cmax mg/l); (Output: Cmin- Cmax mg/l ; removal %)					
	Pollutant	***Canna indica***	***Arundo donax***	***Typha latifolia***	***Phragmites australis***	***Iris pseudacorus***	
Metals	Cr	(<0.001-0.027); (<0.001-0.027)	n.a	(<0.001-2.500); (<0.001-0.750)	(<0.001-2.500); (<0.001-0.646)	(<0.001-0.025); (<0.001-0.040)	24, 26, 27, 30
	Zn	n.a	(0.004); (0.05 - 0.23)	n.a	n.a	n.a	60
	Cu	n.a	(0.01); (0.03-3.37)	n.a	n.a	n.a	60
COD		(20-2669); (2 - 1400)	(28-285); (11-194)	(138-2669); (26-1420)	(51-2669); (4.7-1420)	(329-2669); (1325-27)	24, 26, 27, 28, 30, 29, 31, 140, 34, 35, 41, 48, 42, 86, 85, 83, 77, 188, 132, 140, 18, 100, 135, 137, 85, 127
BOD$_5$		(10-1080); (1-540)	(6-88); (2-37)	(9-1080); (2-760)	(33-1080); (3.89-650)	(20-1080); (9-620)	24, 26, 27, 28, 29, 30, 31, 34, 35, 140, 248, 48, 86, 85, 188, 183, 140, 18, 100, 135, 127, 85
N	No$_3$	(20-60); (0.1-50)	(0.58-34); (<0.1-33.8)	(5-60); (2-47)	(3.45-86.53); (< 0.23 - 52)	n.a	85, 24, 26, 27, 28, 29, 30, 31, 35, 246, 248, 83, 60, 110, 40
	N total	n.a	n.a	(132); (47)	(23.97-126); (48%-93%)	(47); (132)	188, 183, 132, 85
	TKN	(90-230); (62-170)	(0.9-34.2); (0.7-20.5)	(87-160); (57-115)	(43.6-230); (3.7-104)	(33-230); (7-165)	24, 26, 27, 28, 29, 30, 31, 35, 18
	NH$_4$	n.a	24; 84.7%			(45-100); (37-88)	110, 57
P	P total	(0.08 - 0.45); (0.12-0.93)	(0.26-3.00); (0.05-1.00)	(0.08-11.00); (0.30-6.00)	(0.08-17); (0.04-7.00)	(0.15-11); (0.16-6)	24, 26, 27, 28, 29, 30, 31, 35, 85, 86, 188, 183, 127
	PO$^{-3}_4$	(1.5-98.2); (0.7-16.1)	n.a	(0.09-1.4); 100%	(4.4-13.7); (0.4-3.6)	n.a	248, 48, 246, 110, 18
Pharmacs	ibuprofen	n.a	n.a	n.a; (20%-96.2%)	n.a	n.a	72, 80
	Carbamazepine	n.a	n.a	(1µg mL/l); (10%-96.7%)	(1mg/l); (81.5 -86.2 %)	n.a	103, 72, 80
	Atenolol	n.a	n.a	(100 mg/l); (81.5-91.5%)	n.a	n.a	77
	Clofibric acid	n.a	n.a	n.a; (10%-74.5%)	n.a	n.a	72, 80
	Oxytetracycline	n.a	n.a	(1.8-100); (88%)	n.a	n.a	83, 61
SO$^{2-}_4$		n.a	n.a	(78-2206); (12-1890)	(103-265); (90-190)	n.a	27, 26, 30, 29
Solids (SST. SSD)		(10-1500); (0.09-39)	(17-146); (3-91)	(10-1500); 75%	(10- 631); (1-292)	(42-316); (17-21)	24, 26, 27, 28, 30, 29, 31, 140, 34, 35, 248, 48, 86, 85, 83, 188, 183, 140, 100, 18, 127
Herbicides (MCPA)		(3-52.5); (4.3-80.3)	n.a	n.a	n.a	n.a	83
Phenol (Polyphenols)		(3.1-89.1)	n.a	n.a	n.a	n.a	83

Calheiros et al, (2007) showed that *P. australis* had a high accumulation of Cr in its rhizomes with a low translocation content of this metal, indicating that this plant may not be the most suitable to perform phytoextraction since having a high translocation content is a key factor for a plant to have a good phytoextraction capacity. In addition, the accumulation of Cr does not occur in a harvestable part and also, some histotoxic symptoms such as leaf discoloration and necrosis were pointed out. The fact that *P. australis* presents these symptoms raises the doubt if the plant can hold out longer receiving such a concentration of Cr. This would imply the need for constant replacement of the plant, characterizing an expensive and not sustainable process. For this reason, it might be better to give priority to the use of other plants such as *T. latifolia*. This species does not present such a capacity of accumulation and still manages to reach satisfactory results in Cr removal, surviving for a longer time as how it does not reach such levels of toxicity.

No studies proving the capacity of a plant to be a hyperaccumulator were found in the literature research. Only two results close to this characteristic were found. The first one was the study made by Calheiros et al (2007) relating *P. australis* to Cr accumulation. In this case, the accumulation occurred in the rhizome > shoot > leaf. The other one was the use of *A. donax* to phytoremediate Zn and Cu (Costa, 2014). In this lab- scale experiment, it was found that for such concentrations of Zn (16.5 mg/kg - 31.6 mg/kg) and Cu (4.16 mg/kg - 5.83 mg/kg), the maximum enrichment coefficient obtained (0.018 for Zn and 0.026 for Cu) is well below what was defined by Kausar et al (2012) as a minimum concentration that a plant can shows to be considered a hyperaccumulator. However, the bioaccumulation factors obtained demonstrate that the plant has a high capacity to bioaccumulate these metals from wastewater and soil in its tissues without exhibiting phytotoxic effects. It was also observed that in situations of water stress, *A. donax* can accumulate Zn and Cu mainly in the aerial part, making pruning possible. In situations of no water stress, the plant accumulates these metals mainly in the rhizomes.

Cassoni (2016) studied the ability of the plants *S. maritima*, *J. maritimus* and *A. donax* to treat saline wastewater in CW microcosms. The system using the *A. donax* plant had the best ammonia and nitrate removal, with 85% and 68% removal percentage, respectively. We can confirm this fact from table 3, where the percentage of this plant for both factors was satisfactory. Furthermore, it was obtained that all plants survived the experiment, proving to be adaptable to saline environments, but *A. donax* was the least adapted species, presenting low growth. Regarding salt removal, although not in the top 5, *S. maritima* obtained the best result, with a maximum removal of 10.4%.

Regarding wastewater from aquaculture processes, two papers were found receiving saline effluents where one of them compared the CW efficiency in treating saline and freshwater wastewater. Jesus et al (2014) studied a CW mesocosm using *T. latifolia* to treat highly saline effluent (salinity 2.4%) from aquaculture with the goal of reducing salt and nutrient concentrations. The results obtained were 94% removal of NH_4^+, 78% of NO_2^-, 46% of NO_3^- and no output of PO_4^{3-} was detected. Thus, the plant was very efficient in removing ammonia, showing that there was good oxygenation in the rhizosphere region, since the conversion of ammonia to nitrite is an aerobic oxidation process. Furthermore, the transformation process from nitrite to nitrate also benefits from the presence of oxygen. Once again, table 4 shows the efficiency of *T. latifolia* in removing the given parameters.

The other study was conducted, also by implementing CW microcosms with *T. latifolia* (Jesus et al, 2012). The first system received a synthetic freshwater aquaculture simulation (composition: 0.44 mg/l NH_4^+, 3.14 mg/l NO_3^-, 0.36 mg/l NO_2^-, and 1.18 mg/l of PO_4^3) and the other was irrigated with wastewater from a saline aquaculture facility (Composition:

0.25 mg/l of NH_4^+, 18.83 mg/l of NO_3^-, 0.78 mg/l of NO_2^-, and 1.41mg/l of PO_4^{3-}). At the end of the analyses, similar removal results were obtained for both microcosms, with only one difference regarding the higher ammonia removal in the systems that received saline water. In both systems, nitrate removal was low, which can possibly be explained by its high initial concentration, and the percentage of PO_4^{3-} removal increased with time, reaching close to 100% removal. Still on the use of *T. latifolia* in CW receiving saline effluents, Brix et al (2002) found that high values of nitrate and low values of ammonia can limit the growth of this plant in environments like this, which was the case in this work besides the fact that the plant grew in saline environments, which is already a limiting factor for growth because it is a survival strategy of this species, and can return to a very high growth rate when there is excess nutrients or fresh water (Jesus et al, 2012).

Two papers in which CW systems treated wastewater from agribusiness were found. In the first work, CW microcosms were constructed using *P. australis*, receiving an effluent from a piggery doped (or not) with 100 µg/l enrofloxacin and/or 100 µg/l ceftiofur, two of the antibiotics mostly used in this type of industry. There was therefore evaluated the ability of the system to remove three groups of antibiotic resistant bacteria’s (ARBs): Heterotrophic bacteria, Coliforms and *Enterococcus*. In the end of the experiment, Ribeiro (2016) reported that the removal efficiency of ARBs was higher than 51% for Heterotrophic bacteria, than 99% for Coliforms and then 97% for *Enterococcus*. Since these groups of bacteria are antibiotic resistant groups, it could be stated that CWs are a valid alternative for the removal of antibiotic resistance, reducing the risk that pig farm effluent discharge poses to the environment (Ribeiro, 2016). Dordio et al (2013) conducted the second study related to wastewater from agricultural processes. In their study, a CW mesocosm using *P. australis* was performed with the aim of treating olive mill wastewater (OMW), swine wastewater (SW) contaminated with oxytetracycline and water contaminated with the herbicide MCPA. The SW and OMW systems obtained more than 80%. of suspended solids and organic load removal A great result was also obtained in the removal of the antibiotic from saline wastewater, showing the ability of *P. australis* in phytoremediation of pharmaceuticals compounds. Another conclusion was that although the LECA substrate used had great results in the uptake of polyphenols and the pesticide MCPA, the presence of the plants in the system considerably helped the process, because while for an initial concentration of 5 µg/l that the planted system and the control system without plant received, this last one obtained a removal efficiency of this herbicide between 40-56% , while the systems planted with *Phragmites*, obtained a removal of 66 -77%, as shown in table 4.

For the present work it was considered that those papers concerning urban wastewater were only wastewater from municipal wastewater treatment plants where it is considered that the composition of the wastewater is not only domestic water but also from streets and commerce. The results obtained for these systems will be discussed in further where the full-scale systems surveyed will be further detailed. However according to Figure 14 the plants that were used most often in CWs associated to wastewater treatment plants were *T. latifolia*, *P. australis*, and *I. pseudacorus*. Among the surveyed papers, after systems involving the treatment of industrial wastewater, those treating domestic effluents were found to be the most numerous. Of these, 8 papers dealt with the removal of pollutants in a generic way and 8 focused on the removal of pharmaceuticals from domestic wastewater. The plants used in the studies about drugs removal were *P. australis* and *T. latifolia*, highlighting its resistance potential and efficiency in the removal of substances such as ibuprofen (anti-inflammatory and analgesic), carbamazepine (anticonvulsant), clofibric acid (antilipemic), oxitetracycline (antibiotic), atenolol (betablocker and antihypertensive). In these studies, *P. australis* was used to remove carbamazepine, atenolol, and oxytetracycline (Pinto, 2009; Gonçalves, 2009).

Pinto (2009) tested the efficiency of *P. australis* in the removal of atenolol and verified that, although most of the removal was performed in the substrate (LECA), the contribution of the plants was 12 to 14% in relation to the total removal of this pollutant by CW. It was also verified, that the presence of the plants made the removal kinetics significantly faster making possible a shorter retention time of the wastewater in CW. Gonçalves (2009) evaluated the removal of carbamazepine by *P. australis* obtaining similar results to Pinto (2009) for the removal of atenolol, that is faster kinetics, higher removal capacity attributed to the substrate but with better results due to the presence of the plant. However, he adds that there was a reduction in efficiency in the winter period. In the removal of oxytetracycline using *P. australis*, Correia (2010) again observed the relevance of the CW systems substrate in drug removal. In this work *P. australis* allowed to significantly accelerate the removal process, since it was obtained 88% removal of this drug in the planted beds and 67% in the non-planted beds in the first six hours. Moreover, oxytetracycline was not found in the leaf tissue, showing that the drug is either not absorbed or not translocated to the aerial part, or on a rapid metabolization by the microorganisms present in the rhizosphere. It is also possible to consider the ability of the plant to release exudates from the roots, which could catalyze the degradation of this antibiotic.

Gonçalves (2009) also used *T. latifolia* for carbamazepine removal obtaining similar results as *P. australis*. Dordio et al (2007, 2008, 2009,2011) evaluated the removal efficiency of clofibric acid, ibuprofen and carbamazepine from wastewater in CW systems using *T. latifolia*. The use of the plant increased removal rates by 10 to 20% (Dordio et al, 2007) and showed good tolerance to these compounds. Dordio et al, (2009) investigated the removal efficiency considering seasonality and found total removal efficiencies of these same drugs of 96%, with a plant contribution of 2 - 32% which indicates that there is a 26% reduction in efficiency in winter periods. Dordio et al, (2008) evaluated only the removal of clofibric acid, finding results such as no visible signs of toxicity and the ability to handle high concentrations of the drug. Differently, when evaluating the removal of ibuprofen using the same plant, Dordio et al, (2011) obtained results in which the drug impaired the growth of the plant causing oxidative damage, even though the final result was the recovery of the plant showing its ability to regenerate.

As for domestic wastewater treatment, most focused on the removal of organic matter, phosphorus and nitrogen, including one paper dealing with the removal of fecal coliforms. Four innovations were also pointed out being the use of sugar cane, brick and limestone as constituent materials of a CW for phosphorus removal, and the implementation of a polyculture of ornamental plants instead of a monoculture. The results of this last work will be discussed further.

P. australis was used by Pinto (2012) and Mavioso (2010) to remove organic matter using CW systems. While in the second work, it was not concluded that the plants obtained significant results in the removal of COD with respect to the mass loads removed, probably due to the fact that the plants used had not yet completed a vegetative cycle during the course of the study, the first obtained 44% COD removal. In addition, Pinto (2012) also verified the ability of *P. australis* to remove BOD_5 (61%), TSS (65%), and CFU (92 to 97%). Another paper that investigated the efficiency of CWs for fecal coliform removal was Mina et al (2009). However, the results obtained showed that although there was a reduction, according to the legislation, the output densities of the microbial indicators were still high for the discharge of water in bathing areas.

3.4.2 Floating Wetland Islands wastewater treatment

As the FWI systems studied were implemented in the same type of environment, coastal areas influenced by ship activities, the pollutants that both papers aimed to remove were the same. The difference was mainly in the type of platform used and considering some common plants to both systems. Table 5 shows as an example the water body characteristics for one of the studies since they were carried out in the same port.

Calheiros et al (2020) when using *S. perennis*, *J. maritimus*, *P. australis*, H*. portulacoides*, *Spartina versicolor*, *S. maritima*, and *Limonium vulgare*, in a FWI in a port marina, came to conclusion that *S. maritima* and *S. perennis* were the more resistant and more promising plant species to cope with this environment (Table 5).

Table 5: Examples of the water characteristics (minimum and maximum values) found in the surface seawater port marina at Porto de Leixões (Calheiros et al, 2020 a).

	(Minimum, Maximum) values
Parameters	(Calheiros et al, 2020 a)
Salinity (ppt; psu)	(28.1, 34.6)
PO_4^{3-}(mg/l)	(0.07, 0.43)
NH_4^+(mg/l)	(<DL, 0.68)
NO_2^-(mg/l)	(0.08, 0.31)
NO_3^- (mg/l)	(0.93, 2.20)
COD (mg/l)	(134, 630)
TPHs (mg / l)	(<DL, 6)

(DL) detection limit<3.0

3.5 Media associated to constructed and Floating Wetlands systems

3.5.1 Constructed Wetlands substrate

Figure 22 shows the main substrate types used taking as total the sum of the papers that addressed the pilot-scale systems, while Figure 23 shows the substrates used in the full-scale systems.

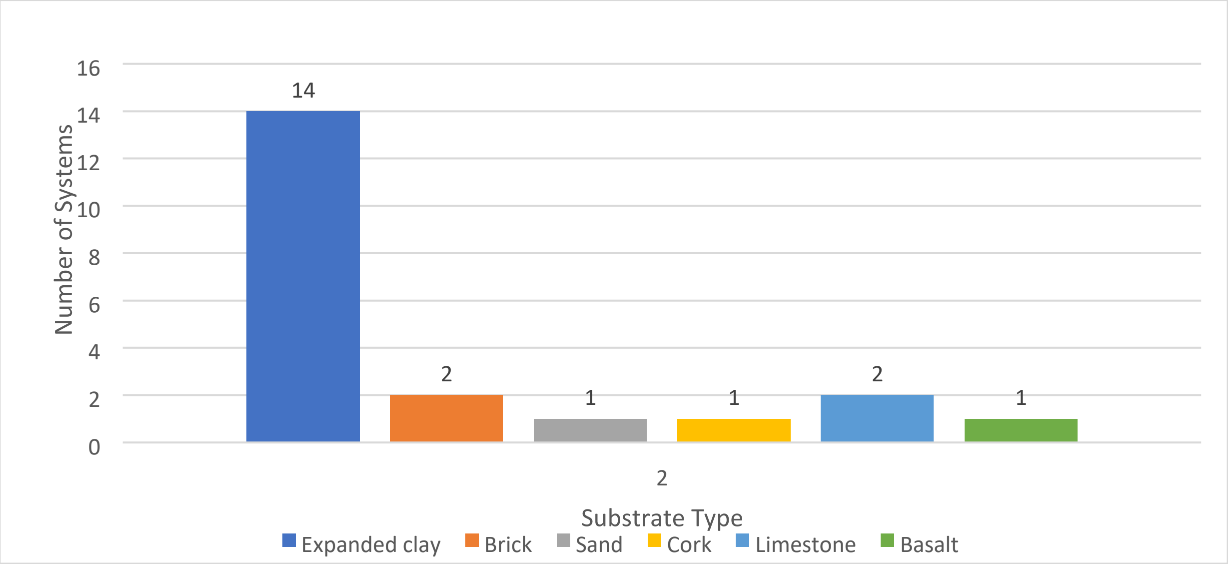


Figure 22: Types of substrates found in constructed wetland pilot-scale systems.

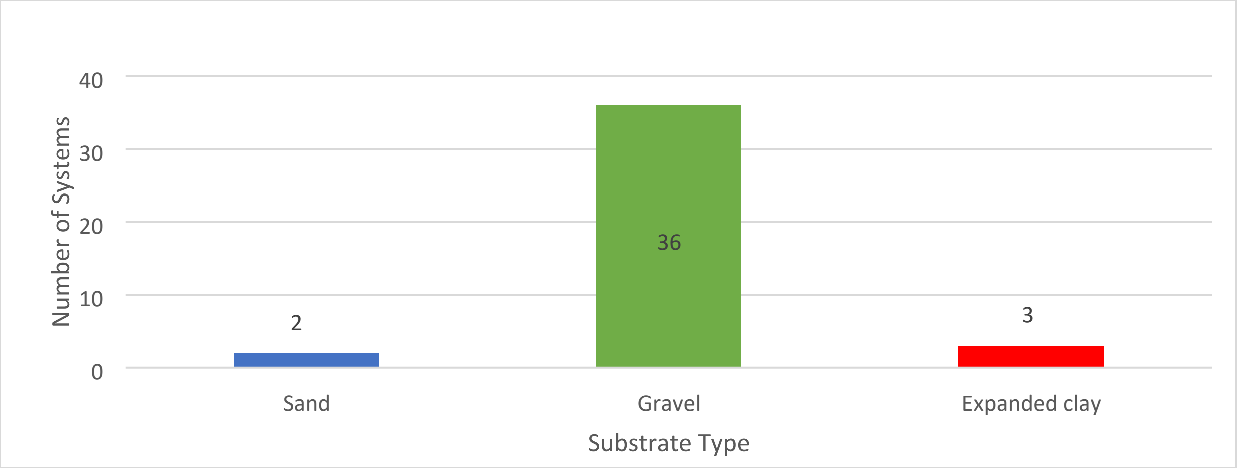


Figure 23: Types of substrates found in Constructed Wetlands real-scale systems.

In relation to the pilot systems, a higher diversity of type of substrate was found. Among these, expanded clay was the most used, perhaps because of its proven efficiency and successive positive results, including among the works found in the bibliographical research done for this work. In addition, although traditional substrates such as sand were used, most of the time, together with expanded clay, other substrates considered more sustainable because they were initially considered as waste, such as brick waste, cork, basalt, and limestone were explored. Limestone scraps and brick were the most used substrates after expanded clay, with two studies each.

First, substrates using expanded clay were shown to be efficient in treating pharmaceuticals, particularly LECA. Pinto (2009) showed the potential for drug removal by this substrate using wastewater contaminated with atenolol. Even the control system with no plants, (retention time of four days), was able to remove almost all of the substance from the system, with 75% removal achieved in 24h. Thus, even if the use of the plant has improved the performance of the system as described above, the substrate by itself already represents a considerable removal capacity, showing its importance. Studies such as Gonçalves (2009) and Dordio et al (2009) also corroborate the efficiency of this substrate in the removal of other drugs such as Ibuprofen, carbamazepine and clofibric acid, obtaining similar results.

Dordio et al (2013) also detected the high capacity of LECA in the removal of veterinary herbicides and antibiotics, showing the success in the uptake of the herbicide MCPA and the antibiotic oxytetracycline. This substrate was also successful in treating winery wastewater, such as the removal of COD (Silva, 2015). Cassoni (2016) when using expanded clay in a CW system to remediate saline wastewater corroborated the result of other works regarding the existence of the capillary rise phenomenon, i.e., saline water rises by capillarity through the pores of the expanded clay, superficially wetting the substrate above the water level with a thin layer of water, which is more susceptible to rapid evaporation. This phenomenon also causes the nutrients present in this wastewater to be better distributed between the contact surfaces of the expanded clays, which has a positive impact on the nutrient removal time and capacity of the CW system.

Mateus et al (2013) tested in different systems the capacity of removal of pollutants from the Filtralite® expanded clay, basalt stones, limestone and brick fragments. As a result, it was obtained that Filtralite® brand clay and limestone brick fragments showed the highest adsorption capacity while basalt stones had the worst performance. The substrate using basalt also obtained the worst results with respect to phosphorus removal from the system, while the others were more successful in the process removing from 15 to 30%. Vaz (2016) when conducting a similar study using the same substrates and calcined eggshell, obtained an average phosphorus removal of 63% for brick substrate, 56% for limestone substrate. However, calcined eggshell had the best result in removing phosphorus from the systems.

Cork substrate (a material that comes from a native tree *Quercus suber*) in Portugal was explored by Calheiros et al (2018) and Gonçalves (2009). Calheiros et al (2018) used cork stoppers as a substrate and that proved to be suitable for plant growth and bacterial communities' establishment when dealing with wastewater from the wine industry. Gonçalves (2009) when comparing the performance of cork with LECA in the removal of wastewater containing the drug carbamazepine observed that, although the results with LECA were very significant, cork showed even better performance. Although the studies point to the fact that cork can be considered as an efficient substrate, studies are still needed on the use of this material in CW systems, especially on hydraulic conductivity.

In most of the papers found, the systems used more than one substrate in the same system, mainly sand and gravel. The explanation for the greater use of these more traditional substrates is probably due to the fact that these systems were implemented in the early 2000's, where the use of other substrates with better absorption without much clogging and with greater capacity to create a healthy biofilm was not yet very common. These systems will be better described in Section 3.7.

3.5.2 Floating Wetlands substrate

According to table 6 it is presented the type of platforms used in the FWI systems found in literature for Portugal. Two types of materials were tested: cork (Calheiros et al, 2020; Campos, 2020) and polyurethane with or without coconut coating (Campos, 2020).

The agglomerated cork platform used by Calheiros et al (2020) was shown to be resistant to the environmental conditions that can be found in a port marina. Some suggestions were made aimed at aiding further research such as using a thicker platform to support the weight of the marine biofouling (Carecho, 2019). In addition, studies on the biofilm have shown the ability of this platform to allow microorganisms establishment.

Campos (2020) when testing the polyurethane-PU foam platform, also observed that this material was resistant to the environmental conditions of the saltwater port marina. In the planting phase, the use of coconut fiber coating allowed for greater plant root attachment. However, during the monitoring period the research found that the coating easily retained sediment.

Table 6: Platform materials used in the Floating Wetland Islands associated to the type of plant species and water typology

Plant specie	water typology	Platform material type	reference
Sarcocornia perennis	Port marine	Cork, Polyurethane (with/without coconut coating)	(Calheiros et al, 2020), (Campos, 2020)
Juncus maritimus	Port marine	Cork	(Calheiros et al, 2020)
Phragmites australis	Port marine	Cork	(Calheiros et al, 2020)
Halimione portulacoides	Port marine	Cork, Polyurethane (with/without coconut coating)	(Calheiros et al, 2020), (Campos, 2020)
Spartina maritima	Port marine	Cork, Polyurethane (with/without coconut coating)	(Calheiros et al, 2020), (Campos, 2020)
Limonium vulgare	Port marine	Cork	(Calheiros et al, 2020)
Inula crithmoides	Port Marine	Polyurethane (with/without coconut coating)	(Campos, 2020)

3.6 Ecosystem services delivered by constructed and Floating Wetlands

Table 7 shows the ecosystem services provided by each real scale CW analyzed in the literature review related to the Portuguese territory. It can be observed that the main ecosystem provided by the full-scale systems studied are water quality improvement, nutrient recycling and removal, and the Habitat promotion for microorganism establishment. However, there was no work mentioning the production of food and biomass for energy production, the increase of richness and/or abundance and/or diversity of birds and related to the mitigation of event-associated flow regime and flood resilience. These services are of great importance especially when it comes to mitigating the effects of climate change and the large consumption of food and fossil fuels. The possibility of conducting studies with this focus in Portugal remains open, since no studies specifying these services were found.

Table 7: Ecosystem services and functions provided by each real scale constructed wetland analyzed in the literature review. The numbers between parentheses means the papers published about the same system.

Ecosystem services	Function	References
Provisioning		
Water	Water reuse for irrigation	204
Ornamental resources	Ornamental and decorative vegetation	41, 43, 44, 45, 46, 48, 42, 40, 33
Regulating		
Air quality	Microclimate effect	45, 46
Water purification	Water treatment	28, 29,30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40 41,43, 44, 45,46,48,42,40,33, 24,37,36, 26, 204, 47, 72, 83, 77, 103, 80, 79, 192, 133, 132, 203, 110,23, 57, 137, 86,85,188,18,137, 99, 183,140,100
Supporting/ habitat		
Biodiversity	Enhancement of biodiversity	41,43, 44, 45,46,48,42,40,33, 24,37,36, 26, 204, 47, 72, 83, 77, 103, 80, 79, 192, 133, 132, 203, 110,23, 57, 137, 86,85,188,18,137, 99, 183,140,100

	Vegetation diversity enhancement	41,43, 44, 45,46,48,42,40,33
	Pollination	41,43, 44, 45,46,48,42,40,33
	Habitat promotion for microorganism establishment	28,31, 33, 43, 44, 46, 47, 48, 52
Nutrient cycling	Nutrient uptake and removal	28, 29,30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40 41,43, 44, 45,46,48,42,40,33, 24,37,36, 26, 204, 47, 72, 83, 77, 103, 80, 79, 192, 133, 132, 203, 110,23, 57, 137, 86,85,188,18,137, 99, 183,140,100
Cultural		
Aesthetic	Ecosystem with aesthetic value integration	41, 43, 44, 45, 46, 48, 42, 40, 33
Recreation and tourism	Recreational site	41, 43, 44, 45, 46, 48, 42, 40, 33
Education	Educational purposes and opportunity for training	41, 43, 44, 45, 46, 48, 42, 40, 33

The systems implemented in municipal treatment plants focused mainly on the ability of these systems to remove pollutants from wastewater. As well as for the systems that treated industrial (tannery and winery wastewater), where the main focus was on the system's ability to remove pollutants through plant uptake and microorganism transformation. Thus, the ecosystem services described in this both types of application were nutrient uptake and removal, presence of microorganism and water treatment. Consequently, even though no specific results could prove that other ecosystem services were provided by systems with these characteristics, Industrial CW could maybe provide the effect on microclimate, biodiversity enrichment of pollinators, air purification and carbon sequestration. It is also possible to suggest the application of environmental education activities to the company's workers, an idea that was not cited in any of the papers, but which can contribute to the ecosystem services that can be generated by the implemented system.

Finally, the system that encompassed the largest number of research exploring the ecosystem services generated was the system implemented in Paço de Calheiros. Studies were carried out on the influence on the microclimate, the presence of biocenosis associated with the substrate, the associations of microorganisms in the rhizosphere of the plants and in the substrate biofilm, and the capacity of removal and recycling of nutrients in warmer and colder seasons of the year. Also, a toxicity study was carried out on lettuce and plants to verify the possibility of reusing the water that comes out of the system for irrigation and for decorative use, respectively. An overview of all the ecosystem services that this system can generate was also published. These studies will be better detailed in Section 3.7.2.

Ecosystem services were as well generated by the FWI systems that were studied (Table 8). They focused mainly on the recycling and uptake of nutrients and pollutants, since one of the main goals of both studies was to access the capacity of FWI to mitigate the water pollution around the port. Biodiversity supports were also provided. The floating mat and plant roots served as habitat and support for various macroinvertebrates, fish and small crustaceans. It was also observed a large association of algae and mussel biofilms in the system. Aesthetic values were provided since it implemented an ecosystem with aesthetic value integration in the port. Educational benefits were also cited since these were scientific studies that were published to the community. Benefits such as carbon sequestration and provision of valuable biomass have not been studied.

Table 8: Ecosystem services provided by each Floating Wetland Island analyzed in the bibliographic review

Ecosystem services	**Function**	**References**
Regulating		
Water purification	Water treatment	50, 55, 53
Supporting/habitat		
Biodiversity	Enhancement of biodiversity	50, 55, 53
	Habitat promotion for microorganism establishment	50, 55, 53
Nutrient cycling	Nutrient uptake and removal	50, 55, 53
Cultural		
Aesthetic	Ecosystem with aesthetic value integration	50, 55, 53
Education	Educational purposes, research and opportunity for training	50, 55, 53

3.7 Case studies in Portugal

3.7.1 Constructed Wetlands in the tannery industry

Two systems applied in field conditions but taking advantage of real operating conditions were found. These two systems were used to treat effluents from tannery industry using subsurface flow CWs. One of the systems was located in the north and the other in the center of Portugal. In the company located in the north, two series of two-stage units were built, with the same characteristics, but one using *P. australis* and the other *T. latifolia* (Figure 24). Both used the same expanded clay substrate (Filtralite®MR). The researchers sought to analyze the potential of each plant as well as to evaluate the microbial dynamics in the root's plants and in the substrate (Calheiros et al, 2009a; Calheiros et al, 2009b).

Among the results found, *P. australis* was the plant that obtained more success when there was more oscillation of the loads applied, although *T. latifolia* also showed satisfactory results. *P. australis* also achieved, through toxicological studies, a high potential to extract and accumulate chromium. Both species showed no visual signs of phytotoxicity. The system proved capable of functioning well when subjected to higher organic and hydraulic loads. The pilot was also able to handle fluctuations in organic loads and feed interruptions without plugging. In the end it was possible to prove the pollutant removal capacity of both plants and the higher efficiency of the two-stage systems.

When performing cluster analysis, it was observed that a diverse and distinct bacterial community was formed in each CW, with each species evenly distributed within each unit. It was noted that possibly the plant type as well as the stage position exerted an important effect on the dynamics of the bacterial communities, while the different hydraulic loads that corresponded to increases in organic matter did not seem to result in changes for these communities. One factor that may have contributed to the establishment of these diverse communities is the interaction during the operation time of plant roots, substrate and microorganisms

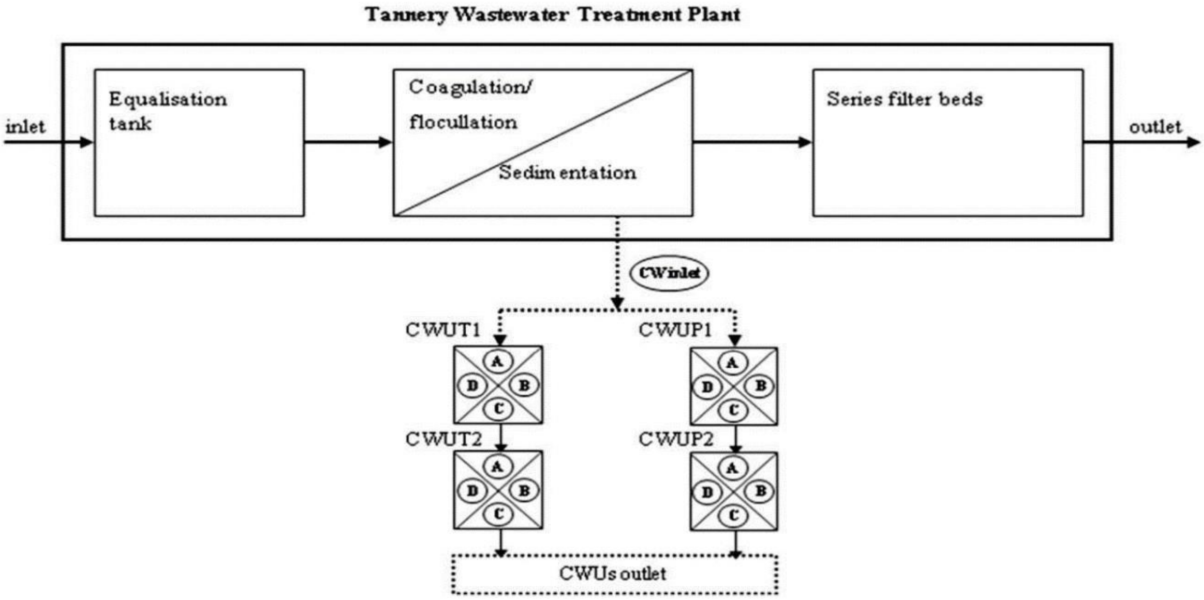


Figure 24: Schematic representation of the constructed wetland units (Calheiros, 2009).

Another study using a similar process was conducted by Calheiros et al (2011) this time receiving wastewater from a Tannery Industry in the central region of Portugal. In this case, the plants used were *A. donax* and *S. fruticosa. A. donax*, although considered an invasive plant, was used due to its abundance in the area around the industry. The system was used for tertiary treatment receiving wastewater from the activated sludge process (Fig.25)

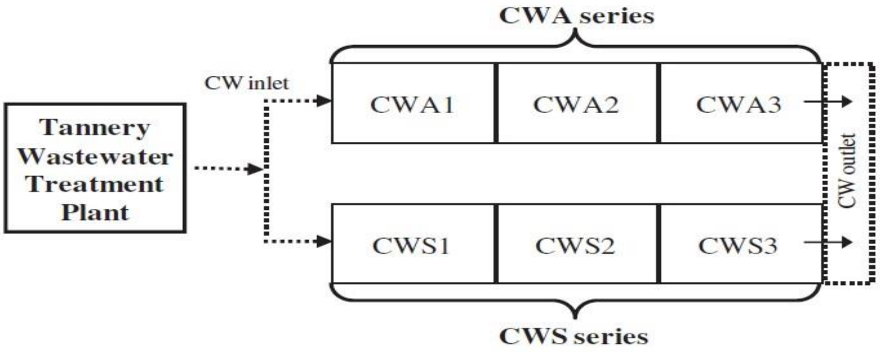


Figure 25: Schematic representation of the constructed wetland units (Calheiros,2011).

The results for the COD and BOD_5 removal varied between 80% and 90% without expressive difference between the two species. Also, there was no difference between the two species. For the removal of TSS the variation ranged from 2% to 30%, and for the other parameters, both systems presented removal capacity in agreement with the Portuguese legislation. In relation to the growth and development of the plants, even though in both species established well without any toxicity sign, *A. donax* was considered as being the most promising plant due to its longer roots and better capacity of growth. Also, a fact to be mentioned was the infestation of *A. donax* for the insect aphids. However, this fact did not cause any problem related to its growth and development.

3.7.2 Constructed wetland in a tourism house

For the purpose of this research a fieldtrip was made to a full-scale CW system located at Paço de Calheiros, as previews described. Besides that, 13 papers were found in the literature research. The use of expanded clay as the system's substrate proved to be suitable for the establishment of the selected plants as well as a good substrate for bacteria biofilm. According to Calheiros et al (2021) the system continues to obtain a satisfactory result since it was implemented, with respect to the removal of COD, BOD_5, TSS, PO_4^{3-}, NH_4^+, NO_3^-, and NO_2^- (Table 9). This study also proved the efficiency of the system in reducing the concentration of *E. Coli* from wastewater that had a typology similar to a domestic wastewater, however with a flow inconstancy due to the variation in the number of guests. Although, according to the seasons of the year, no significant difference was found in the removal of the aforementioned physicochemical parameters, the system presented different results regarding the removal of *E. Coli* in the hot and cold seasons. In the colder periods, the count of *E. Coli* was lower, as expected, corroborating the idea that the development of the bacterial community depends mainly on the seasons of the year and climatic conditions.

Domestic wastewater often has several types of pathogens that can accumulate in plant tissue. Calheiros et al (2016) conducted a study that analyze the presence of endophytic bacteria in *C. flaccida* tissues. In this research was possible to conclude that the genera *Citrobacter, Serratia, Rahnella, Raoultella* and *Pantoeawere* were present in the plant shoots, which can be a risk to human health if these ornamental plants will be used for decorative purposes in flowerpots. In order to clarify this issue, another research was made (Calheiros et al, 2017b) obtaining as result that *E. coli* and *L. monocytogenes* were always below the limit of detection regarding the contact of water with the plants, indicating its safe use for ornamental purposes. Although, *Enterobacteriaceae* were detected in the plants and in the vase water. Moreover, when accomplishing the irrigation of lettuce with the outlet of CW systems, the presence of L*. monocytogenes* and *E. coli* was detected, being observed the occurrence of an insignificant translocation of these bacteria for the plants. However, as the study of bacteria internalization in plants was only performed for *L. monocytogenes*, caution should still be taken when considering the use of wastewater for irrigation purposes (Calheiros et al, 2017b).

Calheiros et al (2019) conducted a study in the abovementioned CW system indicating the phylogenetic affiliation of AMF communities colonizing the roots of three of the plants used in the polyculture (*C. indica, C. flaccida*, and *W. borbonica*). In the case of *C. indica* the most commonly found strain was *Glomus sp.* and in the cooler climates *Acaulospora sp.* was also present. *Glomus sp.* was also found colonizing the roots of *C. flaccida* during the lower temperatures, while in warmer seasons, the strain *Rhizophagus sp.* was outstanding. *W. borbonica*, on the other hand, showed affinity with *Acaulospora sp.* in the cooler seasons and with *Rhizophagus sp.* in the warmer ones.

The plants that performed better in the CW system were *C. flaccida* and *C. indica*, being the plants that dominated the system. *Zantedeschia aethiopica* was able to survive in the system but not proliferate. *Agapanthus africanus* flourished in the first few weeks that the system was in operation but was unable to sustain itself for long, probably due to its slow growth in comparison with the other plant species. Similarly, the plant *Watsonia borbonica* also had its presence inhibited by the two dominant plants, being only present in the system boarders.

Calheiros et al (2021) also showed the difference in flowering of plants during the cooler and warmer seasons. Figure 26 shows the visual difference of the system according to the seasons. Table 8 presents some physicochemical results from the inlet ad outlet obtained in studies carried out in Paço de Calheiros (Calheiros et al, 2015, 2019 and 2021). As we can see over the years the system continued to show good efficiency in removing the physicochemical parameters below.

Furthermore, Calheiros et al, (2017) evaluated other ecosystem service, being the microclimate effect caused by the CW system. It was concluded that the inclusion of this biosystem represented a means of lowering the surface temperature, influencing the surrounding environment, and to some extent having an impact on the heat island effect, being able to be used as a tool for environmental management. The presence of plants and water (such as ponds present in the enclosure), in general, causes the surface temperature to decrease when compared to its surroundings (sidewalk or soil).

Figure 26: Constructed wetland at a tourism house during the cold seasons (left) and warmer seasons (right) (Calheiros et al, 2021)

In order to make an overview of the various ecosystem services generated through the CW system implemented in Paço de Calheiros, another study was conducted. Calheiros et al (2018 a), highlighted that in relation to provisioning services, the system can generate water for reuse, raw material such the plants biomass, and ornamental sources providing flowers for decoration. In relation to regulating services, the system can generate improved water quality due to the greater presence of plants, climate regulation due to carbon sequestration and temperature regulation at a microclimate level, pollination since the plants have prominent flowers, and water purification due to the removal of pollutants from the wastewater done by the system. Other services that this system provides to the ecosystem are in relation to the supporting and habitat services, providing biodiversity, support, and nutrient cycling. Finally, the system still generates cultural services such as aesthetic, recreational for the guests, and educational, since school visits are made to the system, pedagogical activities are applied, and a large number of published works regarding the CW of Paço de Calheiros are carried out.

Table 9: Physicochemical parameters assessment associated with the constructed wetland performance carried out at a tourism unit- Paço de Calheiros

			(Inlet /Outlet) Concentration	**Limit**
Parameters	**Calheiros et al, 2015**	**Calheiros et al, 2019**	**Calheiros et al, 2021**	**Discharge (Decret-Law n.o 236/98)**
COD (mgO$_2$/l**)**	(20-1467)/ (3-78)	(274-577)/ (80-112)	(385-691)/ (11-136)	150
BOD$_5$ (mgO$_2$/l**)**	(10-480)/ (2-30)	(155-220)/ (55-60)	(165-281)/ (7-38)	40
TSS (mg/l)	(10-1500)/ (3-39)	(150-220)/ (8-10)	(66-287)/ (6-14)	60
PO$^{-3}_{4}$- (mg/l)	(1.5-98.2)/ (0,8-10.4)	(6.5-9.10)/ (1.75-3.05)	(15.75-28.75)/ (3.47-8.39)	10; 3 (in waters feeding lakes or reservoirs) 0.5 (in ponds or lagoons)
NH$_4$+ (mg/l)	(2.7-75.3)/ (1.9-41.9)	(9.35-14.48)/ (1.37-1.81)	(19.16-60.22)/ (7.37-27.07)	10
NO$_2$- (mg/l)	(0.1-7.4)/ (0.1-0.3)	-	(0.31-2.18)/ (0.10-0.14)	n.a
NO$_3$- (mg/l)	(0.1-37)/ (0.1-9.4)	(0.61-1.00)/ (0.06-0.39)	(3.42-21.26)/ (0.76-5.00)	50

n.a= not available.

In order to make an overview of the various ecosystem services generated through the CW system implemented in Paço de Calheiros, another study was conducted. Calheiros et al (2018 a), highlighted that in relation to provisioning services, the system can generate water for reuse, raw material such the plants biomass, and ornamental sources providing flowers for decoration. In relation to regulating services, the system can generate improved water quality due to the greater presence of plants, climate regulation due to carbon sequestration and temperature regulation at a microclimate level, pollination since the plants have prominent flowers, and water purification due to the removal of pollutants from the wastewater done by the system. Other services that this system provides to the ecosystem are in relation to the supporting and habitat services, providing biodiversity, support, and nutrient cycling. Finally, the system still generates cultural services such as aesthetic, recreational for the guests, and educational, since school visits are made to the system, pedagogical activities are applied, and a large number of published works regarding the CW of Paço de Calheiros are carried out.

3.7.3 Constructed Wetlands integrated in domestic wastewater treatment plants

In the literature, studies were found that analyzed the implementation costs and performance of 37 WWTP that had a CWs as part of their wastewater treatment. These systems are listed in the table 10. An attempt was made to contact each system bellow, however only 5 systems responded the emails with currently information.

Table 10: Constructed Wetlands integrated in domestic wastewater treatment plants in Portugal

System	**References**	**Currently info.**
Travanca do Mondego – Penacova	Galvão and Matos (2004)	No information
Porto da Raiva – Penacova	Galvão and Matos (2004)	No information
Oliveira Mondego – Penacova	Galvão and Matos (2004)	No information
Coiço – Penacova	Galvão and Matos (2004)	No information
Cunhedo – Penacova	Galvão and Matos (2004)	No information
Silveirinho – Penacova	Galvão and Matos (2004)	No information
Cruz do Soito – Penacova	Galvão and Matos (2004)	No information

S.Paio Mondego – Penacova	Galvão and Matos (2004)	No information
S. Pedro d'Alva – Penacova	Seco (2008), Duarte et al (2010 a), Duarte et al (2010 b)	**Still working**
Meimão – Sebugal	Galvão and Matos (2004)	No information
Amiais – Sebugal	Galvão and Matos (2004)	No information
Galifosa – Viseu	Galvão and Matos (2004)	No information
Lutosa- Viseu	Galvão and Matos (2004)	No information
Ribafeita – Viseu	Galvão and Matos (2004)	No information
Cantanhede	Galvão and Matos (2004)	No information
Barroca D`Alva – Alcochete	Mendes (2010); Botequilha (2013), Seco (2008), Duarte et al (2010 a), Duarte et al (2010 b)	**Still working**
Boaventura – Madeira	Seco (2008), Duarte et al (2010 a), Duarte et al (2010 b)	No information
Aranhas – Penamacor	Seco (2008), Duarte et al (2010 a), Duarte et al (2010 b)	No information
Anobra – Condeixa	Seco (2008), Duarte et al (2010 a), Duarte et al (2010 b)	No information
Gonçalo – Guarda	Seco (2008), Duarte et al (2010 a), Duarte et al (2010 b)	No information
Rossas – Vieira do Minho	Seco (2008), Duarte et al (2010 a), Duarte et al (2010 b), Oliveira (2007)	No information
Salamonde 2 – Vieira do Minho	Seco (2008), Duarte et al (2010 a), Duarte et al (2010 b), Oliveira (2007)	No information
Salamonde 1 – Vieira do Minho	Seco (2008), Duarte et al (2010 a), Duarte et al (2010 b), Oliveira (2007)	No information
Salvador – Penamacor	Seco (2008), Duarte et al (2010 a), Duarte et al (2010 b)	No information
Penedo – Vila de Rei	Seco (2008), Duarte et al (2010 a), Duarte et al (2010 b)	No information
Adiça – Tondela	Seco (2008), Duarte et al (2010 a), Duarte et al (2010 b)	No information
Vale – Tondela	Seco (2008), Duarte et al (2010 a), Duarte et al (2010 b)	No information
Póvoa das Forcadas – Carregal do Sal	Seco (2008), Duarte et al (2010 a), Duarte et al (2010 b)	**Still working**
Malavado – Odemira	Seco (2008), Duarte et al (2010 a), Duarte et al (2010 b), Galvão (2009)	**Still working**
Castelejo – Santa Comba Dão	Seco (2008), Duarte et al (2010 a), Duarte et al (2010 b)	No information
Erada – Covilhã	Seco (2008), Duarte et al (2010 a), Duarte et al (2010 b)	No information

Casal de Frade – Arganil	Seco (2008), Duarte et al (2010 a), Duarte et al (2010 b)	No information
Grada – Mealhada	Seco (2008), Duarte et al (2010 a), Duarte et al (2010 b)	**Still working**
Lousal – Grândola	Seco (2008), Duarte et al (2010 a), Duarte et al (2010 b)	No information
Ruivães	Oliveira (2007)	No information
Fataca	Galvão (2009)	No information

Galvão et al (2004) pointed out 14 domestic water treatment plants in small Portuguese communities. These communities generally presented as water treatment systems individual septic tanks, not always designed and maintained in the most appropriate way, and in some situations, the prolonged use of this type of final disposal causes negative environmental impacts, which should be minimized or completely eliminated. The WWTPs that were pointed out in this study are located in the North and Center regions of Portugal and refer to populations of less than 2,300 inhabitants. Thus, the quantification of the technical and economic performance of the following WWTP that presented CWs systems in association with septic tanks or Imhoff tanks were analyzed: In the municipality of Penacova (Travanca do Mondego, Porto da Raiva, Oliveira Mondego, Coiço, Cunhedo, Silveirinho, Cruz do Soito and S.Paio Mondego), in the municipality of Sabugal (Meimão and Amiais), in the municipality of Viseu (Galifonge, Lutosa and Ribafeita) and finally in the municipality of Cantanhede. No information regarding the plants and the substrates used were found in this research.

As one of the objectives of the work of Galvão et al (2004) was to compare WWTP that used CW as secondary treatment, with those that used other types of secondary treatment, at the end of the work it was concluded that one of the main advantages of the use of Constructed Wetlands systems concerns the reduced associated energy costs, especially relevant for populations less than 500 inhabitants. However, during the literature search done for the purposes of this work, no other information related to these systems was found. E-mails were also sent to the councils of the municipalities and no responses or updated results were obtained.

Seco (2008) and Duarte et al (2010 a, 2010 b) have conducted studies referring to 20 CWs that were/are in operation in WWTPs in Portugal as pointed out by the table 10. Seco (2008) studied the main criteria and sizing methods, proposed by different authors, for horizontal subsurface flow CW. In addition, they analyzed the average per capita bed surface area used in these treatment units, as well as the per capita costs of installation and costs/m^2 per bed. About the average areas per capita occupied by the beds, was obtained an average area of 4.5 m^2 per inhabitant and 1.9 m^2 per inhabitant, respectively. The average cost of installation was also calculated, being 246 euros per inhabitant. When comparing the cost and the area occupied by the treatment bodies of these systems with those using percolator beds, it was concluded that the installation cost was about 10% lower.

Duarte et al (2010a; 2010b) focused on making an analysis of unit costs by evaluating their efficiency in the main wastewater quality parameters. In addition, the analysis identifies the main operational problems of these systems, suggesting some mitigating measures and/or corrective measures to improve their operation, as well as the rationalization of their procedures for accurate monitoring. The great majority of the systems they analyzed were using *P. australis*, with the exception of Arganil, which used *Junco*, Condeixa which used *I. pseudacorus* and *T. latifolia*, Covilhão and St. Comba Dão which used *T. latifolia* and the Madeira system which used *I. pseudacorus* and *Juncus*. Still, of the 20 systems analyzed, almost all presented problems with clogging, with the exception of Rossas, Salamonde 1 and 2, Odemira, Madeira and Alcochete. On the other hand, the systems of Aranhas, Arganil, Covilhã, Salvador and St. Comba Dão besides the clogging presented a concentration of suspended solids above the allowed level, and the systems of Guarda and St.Comba Dão besides the clogging, presented a bad odor.

The main operational problems pointed out by Duarte et al (2010a; 2010b) were mainly related to the clogging of the beds, which result in loss of treatment efficiency since the pores of the bed are no longer free to allow percolation of the effluent to be treated. It was also pointed out the absence of an adequate monitoring plan to control the quality of treated effluents in the various stages of the treatment process and deficiencies in the development of planted macrophytes, resulting in a growth rate higher than the top of the bed. This fact ends up favoring the predominant occupation of the rest of the bed by invasive species. Also related to operational problems, Seco, (2008) showed that only 30% of the systems analyzed had no operational problems. About 25% of the systems do not carry out maintenance of the systems. Furthermore, 70% of the systems showed clogging of the beds, 25% allowed the passage of solids into the beds and 15% detected the presence of odors. His research also pointed out the big problem caused by the connection of clandestine effluents.

Oliveira, (2007) analyzed some points referring to 4 WWTPs in Portugal: Salamonde 1, Salamonde 2, Ruivães and Rossas. The analysis of the main physicochemical parameters, flow measurement, hydraulic efficiency tests and observation of the evolution of the plants were carried out. Furthermore, Galvão et al (2009) using ICZM - Integrated Modelling of Constructed Wetlands, studied the hydraulic and environmental behaviors of two Constructed Wetlands, in the variant of subsurface horizontal

One of the results obtained by Oliveira (2007) was that at the time the most commonly detected problems were: Design errors (absence of effluent characteristics, use of only one bed with all the necessary area, preventing maintenance operations and the use of reactors in series or in parallel), construction errors (absence of construction and inspection protocols, septic tanks with sloping, cracked or insufficiently waterproofed bottoms; beds with incorrect bottom slope, poor application of waterproofing material, use of poor quality construction materials, including porous media) and maintenance and operation errors (absence of an operation start-up protocol, absence of a maintenance and operation protocol, neglect and abandonment of the CW and invasion of weeds which, in a short time killed the macrophytes).

Oliveira (2007) also pointed out the main costs related to the implementation of a CW system in 2007. The values indicated were: geotechnical prospection (10.000 €), land clearing (2.500 €/ha), excavation and earthworks (4 €/m^3), sealing of beds (15 €/m^2), porous medium (20 €/m^3), inlet and outlet structures, piping (8 €/m) and plants (2 €/m^2). In addition to these values, a cost of between 8.000 and 35.000 Euros for primary treatment and 1200 Euros/year for maintenance services were also included in the calculation.

Regarding the analyzed WWTPs, the Rossas system presented a very good general functioning, and only small details were suggested for the improvement of the system such as reducing the infiltrated rainwater flow to avoid flooding the bed. Regarding the Salamonde 1 system, as it presented a bad odor at the time, it was advised to cover the grating area and to evaluate the possibility of using an aeration pipe in the septic tanks. In relation to Salamonde 2 system, due to its presented overflows in times of heavy rainfall, it was suggested the implementation of a rainwater drainage ditch with a grille. The Ruivães system was the one that presented the most problems. It was suggested that in order to avoid overflows and flooding, the distribution of the flow through the beds should be corrected by adjusting the crest of the spillways, reducing the flow of infiltrated rainwater and rehabilitating the infiltration system. In order to avoid odors, an aeration pipe should be implemented in the septic tank. In addition to these measures, other measures were also suggested, such as analyzing the structural and geotechnical stability of the beds, rebuilding the retaining wall near the infiltration zone of the beds, redesigning access to the WWTP, with a wider walkway equipped with lateral protections, and improving the fence in the entrance area by placing a door.

In the points below it is possible to find the description and data from the WWTPs that answered to the contact made, together with previous data found in literature.

3.7.3.1 WWTP Barroco D`Alva

Another WWTP that was found in the literature was Barroc D`Alva, present until today in the municipality of Alcochete. As said in the Alcochete sanitation system website, this system is working since 2001 being one of the first to be built in the Setúbal district (https://www.cm-alcochete.pt/viver/agua/rede-de-saneamento, accessed day 20/07/2021 at 19:30). Since then, it presents very favorable results regarding the treatment values of domestic wastewater from Barroca d'Alva, which includes, in addition to housing, a rural hotel and a film studio. Mendes (2010) pointed out that this horizontal subsurface constructed wetland system that was built by the company ETARPLAN, S.A., at the time served a population of 500 inhabitants. The plant used was *P. australis*, and the substrate used was a Kickuth medium with a composition of 71.67% coarse sand, 20.76% fine sand, 5.81% silt and 1.75% clay. This study not only analyzed the capacity of the system to remove pollutants, but also analyzed the calculated daily evapotranspiration, which can influence the flow rate but also can serve to show the influence of the system on the climate regulation of the local microclimate.

Botequilha (2013), also conducted a study regarding the WWTP of Barroco D`Alva. This work aimed to study the effect of seasonal variation on pollutant removal during spring and summer in CW of Barroca D`Alva. This work also studied the buffering capacity of CW after a series of organic load rate increments in the wastewater. As a result, it was obtained that the system showed better removal efficiency during the summer. It was also concluded that despite the oversizing of the CW, it proved to have high pollutant removal efficiency, since the pollutant concentrations at the discharge site were below Portuguese standards. At the end of the study, it was proven that the system has high removal efficiency in the treatment of wastewater from decentralized rural areas. Its performance after 12 years of implementation, with very low maintenance, shows that CW meets the requirements to be considerably the most suitable technique for this rural area.

When contacting the entity responsible for the system, the response was that the system is still functioning and that the plants have good survivability in the system. The results of the most recent water analysis were also shared by the company to assist this research. The physicochemical characteristics of the water from the inlet and outlet of the system found in the years 2008,2013 and 2021 are highlighted in table 11. It is possible to see that all the discharge values are in accordance with the discharge limits designated by Directive 88/347/EEC of 16 June. The data from 2021 was sent by email by the Alcochete municipality.

Table 11: Physicochemical water characteristics from the WWTP Barroco D`Alva inlet and outlet within the years 2008 (Seco,2008), 2013 (Botequilha,2013) and 2021 (Alcochete municipality: disu.cqa@cm-alcochete.pt).

Parameters	**Seco, (2008) (inlet); (outlet)**	**Botequilha, (2013) (inlet); (outlet)**	**2021 (inlet); (outlet)**	**Limit discharge (Decret-Law n.o 236/98)**
COD (mgO_2/l)	(29.7- 77.3); (2.2-9.6)	(28.8-258.3); (14.6-38.5)	n.a; 43	150

BOD$_5$ (mgO$_2$/l)	(31- 80); (2-10)	(16.9-143.9); (1.6-4)	n.a	40
TSS (mg/l)	(62); (23)	(20.5- 109.2); (4 - 55.1)	26; <5 (LQ)	60
P total (mgP/l)	(3); (2)	(0.86-11.4); (0.54-3.6)	7; 1,6	10; 3 (in waters feeding lakes or reservoirs); 0.5 (in ponds or lagoons)
N total (mgN/l)	(61); (34)	(1.8- 49.8); (0.52-2.1)	45; 4.3	15
pH	(6.5l1- 7.15); (6.46- 6.78)	(7.1- 7.3); (7.3-7.9)	7.6; 7.7	6.0-9.0

LQ= Is the lowest concentration of the compound/substance that can be determined with an acceptable level of accuracy and precision and can be obtained from LD studies (lowest non-zero concentration that can be reported with 99% confidence, involving all the steps of the analytical method, from sample preparation to instrumental analysis).; n.a= not available

It is possible to observe the similarity of results found in the efficiency of this system between the data present in Seco's, (2008) research and the physicochemical information recently collected. A highlight goes to the nitrogen values, which 13 years later continue to show values above the discharge limit.

3.7.3.2 WWTP Carregal do Sal

The CW system service present at the Carregal do Sal WWTP began in 1998. Its total area is 655 m2 being the planted area of 388m2 containing *P. australis*. The system is formed by two beds and in 2010 the analyses were performed quarterly. The main problem detected was clogging. (Duarte et al 2010a; 2010b) No information was found on the cost of this system, on the substrate used as well as no studies on the diversity of microbial communities in the system and ecosystem services. Contact was made with this WWTP in June/2021 that responded indicating that the system is still in activity, with all the plants still alive. They also sent a bulletin with information about the recent physical-chemical data present in the last outlet samples (April /2021) as shown in table 12.

Table 12: Physical and chemical parameters present in the urban wastewater outlet from Carregal do Sal WWTP (April /2021) Source: Carregal do Sal City Hall (geral@cm-carregal.pt)

Test/Method	**Result**	**Unit**	**Limit Value** **(Decret-Law n.o 236/98)**
pH PT-MET-19 (2018/11/06)	7.0	Sorensen Scale	6-9

NH4+ Total PT-MET-49 (2017/09/26)	31	mgNH_4^+/l	10
BOD_5 PT-MET-27 (2017/04/05)	18	mgO_2/l	40
COD PT-MET-32 (2016/09/02)	57	mgO_2/l	150
Total P PT-MET-120 (2021/09/06)	4.2	mgP/l	10; 3 (in waters feeding lakes or reservoirs); 0.5 (in ponds or lagoons)
TSS PT-MET-21 (2014/05/06)	22	mg/l	60
N total PT-MET-70 (2018/06/15)	27	mgN/l	15

According to the data it can be seen that the pH of the water was neutral and that the parameters are in accordance with the emission limit values for wastewater discharges according to the legislation, with the exception of the nitrogen values (ammoniacal nitrogen and total nitrogen) which were above the required values (10 and 15ml/l, respectively).

3.7.3.3 WWTP Grada - Mealhada

The CW present at the Mealhada WWTP was implemented in the 2000s with a total area of 1.000m^2 being 350m^2 of area planted with *P. australis*. This system contained one bed and its implementation cost was 38.513.00 euros. In 2010 the system was analyzed monthly (Duarte et al, 2010a; 2010b). No studies were found on the substrate used as well as on the diversity of microbial communities in the system and ecosystem services. The WWTP is still in operation and all the plants survived, according to information received after contact with this WWTP in July/2021. Information was sent as shown in table 13 (inlet) and table 14 (outlet), containing results on the recent physical-chemical data present in the last inlet and outlet samples (June/2021).

Table 13: Physico-chemical parameters present in the urban wastewater outlet (inlet) from the WWTP of Grada-Mealhada. (June /2021)SourceE: Municipality of Mealhada (*aguas@cm-mealhada.pt*).

Test/Method	**Result**	**Unit**	**Limit Value (Decret-Law n.o 236/98)**
pH PT-MET-19 (2018/11/06)	6.5	Sorensen Scale	6.0-9.0

BOD$_5$ PT-MET-27 (2017/04/05)	920	mgNH$_4^+$/l	40
COD PT-MET-32 (2016/09/02)	6330	mgO$_2$/l	150
Total P PT-MET-120 (2021/09/06)	22	mgO$_2$/l	n.a
TSS PT-MET-21 (2014/05/06)	1200	mgP/l	60
N total PT-MET-70 (2018/06/15)	120	mg/l	15

n.a= not available.

Table 14: Physicochemical parameters present in the urban wastewater outlet (outlet) from the WWTP of Grada-Mealhada. (June /2021) Source: Câmara Municipal de Mealhada (*aguas@cm-mealhada.pt*).

Test/Method	**Result**	**Unit**	**Limit Value (Decret-Law n.o 236/98)**
pH PT-MET-19 (2018/11/06)	7.5	Sorensen Scale	Sorensen Scale
BOD_5 PT-MET-27 (2017/04/05)	18	$mgNH_4^+/l$	40
COD PT-MET-32 (2016/09/02)	41	mgO_2/l	150
Total P (2021/09/06)	11	mgO_2/l	n.a
TSS PT-MET-21 (2014/05/06)	10	mgP/l	60
N total PT-MET-70 (2018/06/15)	85	mg/l	15

n.a= not available.

According to the data presented it can be observed that the parameters are in accordance with the legal requirements with the exception of the values for total nitrogen that are above the discharge limits. The figure 27 below shows a visual perspective of the system in two different seasons being "A" taken in January 2020 (winter) and "B" June 2021 (summer). It can be seen that, although the appearance of the plantation is very different for each climatic condition, this does not mean any problem with regard to the development of the plants that have maintained their vigor.

Figure 27: Photos of the WWTP of Grada Mealhada. (A) The system during the winter season. (B) The system during the summer season. Source: Municipality of Mealhada (aguas@cmmealhada.pt).

3.7.3.4 WWTP Malavado – Odemira

The WWTP CW in Odemira was implemented in 2001 and its total area is 1.360m^2 of which 714m^2 is planted with *P. australis*, which has been performing very well up to the present day. This system was formed by two beds and its implementation cost was 87040.00 euros with annual analysis frequency not presenting any type of problem. (Duarte et al, 2010a; 2010b). No studies were found on the diversity of microbial communities in the system and ecosystem. Information was sent as shown in table 15 (inlet) and table 16 (outlet), containing results on the recent physical-chemical data present in the last inlet and outlet samples (July/2021). It can be seen that the physical chemical parameters are in accordance with the levels considered acceptable by legislation.

Table 15: Physical and chemical parameters present in the urban wastewater (inlet) from the Malavado Oldemira WWTP. Source: Odemira Municipal Council (aguas@cm-mealhada.pt). n.a. = not available

Parameter/Procedure (Inlet)	**Result**	**Unit**	**Limit Value (Decret-Law n.o 236/98)**
pH MI-24-006 ed. (Potentiometry)	7.4 (21ºC)	Sorensen Scale	6-9
BOD$_5$ MI-24-024 ed.3 (Oxygen Specific Electrode)	19	mgNH$_4^+$/l	40
COD MI 24-021 ed.1 (Molecular Absorption Spectrophotometry Microtest)	101	mgO$_2$/l	150
Oil and Fat MI 24-059 ed.11 (Infrared Spectrometry)	<5 (LQ)	mgO$_2$/l	15
TSS EN 872:2005 (Gravimetry)	7	mgP/l	60
Total P MI 24-089 ed1	29	mg/l	10; 3 (in waters feeding lakes or reservoirs); 0.5 (in ponds or lagoons)
N total MI 04-148 ed. 0	n.a.	mgN/l	15

LQ= Is the lowest concentration of the compound/substance that can be determined with an acceptable level of accuracy and precision and can be obtained from LD studies (lowest non-zero concentration that can be reported with 99% confidence, involving all the steps of the analytical method, from sample preparation to instrumental analysis).; n.a= not available.

Table 16: Physical and chemical parameters present in the urban wastewater outlet (inlet) from the Malavado Oldemira WWTP Source: Odemira Municipal Council (aguas@cm-mealhada.pt).

Parameter/Procedure (Outlet)	Result	Unit	Limit Value
pH MI-24-006 ed. (Potentiometry)	6.7(21ºC)	Sorensen Scale	6-9
BOD_5 MI-24-024 ed.3 (Oxygen Specific Electrode)	n.a.	$mgNH_4^+/l$	40
Total P (mg P /L) MI 24-089 ed1	18	mgO_2/l	10; 3 (in waters feeding lakes or reservoirs); 0.5 (in ponds or lagoons)
COD MI 24-021 ed.1 (Molecular Absorption Spectrophotometry Microtest)	n.a	mgO_2/l	150
Oil and Fat MI 24-059 ed.11 (Infrared Spectrometry)	61	mgP/l	15
TSS (mg/L)EN 872:2005 (Gravimetry)	n.a.	mg/l	60
N total (mg N /L) PT-MET-70 (2018/06/15)	n.a.	mgN/l	15

LQ= Is the lowest concentration of the compound/substance that can be determined with an acceptable level of accuracy and precision and can be obtained from LD studies (lowest non-zero concentration that can be reported with 99% confidence, involving all the steps of the analytical method, from sample preparation to instrumental analysis).;n.a= not available.

3.7.3.5 WWTP S. Pedro d'Alva – Penacova

This CW was built in 2001 and has a total area of $2040m_2$ with a floor area of $1860m_2$ planted with *P. australis*. There are two beds, and the cost is €97266.00. It also presented problems of clogging, although not very frequent, being the analysis period quarterly. This system receives urban effluents, i.e., domestic water, rainwater and water from clandestine connections. After pruning the plants, the biomass was used in the fertilization of the woods, according to Seco, (2008). The data on the physical-chemical parameters received refer to different annual periods, namely: 2008, 2010 and 2021.The data from 2021 was sent by email by the São Pedro D'Alva municipality.

It is possible to see from table 17 that the results have changed significantly from the first two years to the current results. In 2008 and 2010, the values of BOD_5 were above the required limits. However, all the values sent for the year 2021 are in accordance with the requirements of the legislation. Probably some change may have been made over the years in order to optimize the system.

Table 17: Physicochemical water characteristics from the WWTP S. Pedro D'Alva inlet and outlet within the years 2008 (Seco,2008), 2010 (Duarte et.al,2010) and 2021(São Pedro D'Alva municipality).

Parameters	**Seco (2008) (inlet; outlet)**	**Duarte et.al, 2010 (inlet; outlet)**	**2021 (inlet; outlet)**	**Limit Value**
CQO (mgO$_2$/l)	1009; 318	937; 123	n.a; < 15	150
BOD$_5$ **(mgO$_2$/l)**	97;33	554; 42	n.a; < 10	40
TSS (mg/l)	62;23	290; 22	n.a; < 5.0	60
P total (mgP/l)	3;2	12; 7	n.a; 7	10; 3 (in waters feeding lakes or reservoirs); 0.5 (in ponds or lagoons)
N total (mgN/l)	61;34	78;48	n.a; 7	15
pH	7.4; 7.2	n.a	n.a; 6.9	6-9

n.a. = not available

Following a forest fire that affected the system, it was necessary to replace the damaged plants with others, which led to the replanting of the CW systems. The data presented was collected before the fire that occurred after June 2021 and we did not obtain data regarding the total damage suffered after the accident.

3.8 Floating Wetland Island implementation at Leixões Port

The FWI system that was implemented in Leixões port was visually monitored for 3 months. Figure 28 show the visual aspect of the island in the months of June and August. It can be seen that the plants needed time to adapt, as the aerial part of the plant was not yet developed. In addition, a large number of algae started growing in association with the base of the FWI was observed (figure 29). Therefore, green algae could be found at the edge of the system and, in this time, the association of mussels to the system was not observed, at least so far, which can be considered a positive point, since it contributes to the non-sinking of the system. In the first FWI, one plant vases were lost due to a cut in the vessels near the seal that connect the vase to the platform, or to the improper sealing of this seal as for the second FWI, the 3 vases were lost.

Figure 28: Visual aspect of the floating island in the months of June and August.

Figure 29: Mussels and algae attached to the Floating Wetland Island platform.

One of the objectives of the application of this FWI in the present work was to access the difficulty levels of the application of this system. The system was built in a little less than 3 hours in the same day, showing to be of easy implementation and can be applied by anyone.

4 Conclusion and future work

The present thesis aimed, through bibliographic research combined with field observation, to contribute to the knowledge and experiences generated in recent years about the use of phytoremediation (using CWs and FWIs) for wastewater treatment in Portugal. Thus, it can be concluded that there is a legitimate interest in encouraging the use of these sustainable solutions, including in the academic field. The data found in the literature allows us to assess that:

- The research identified that more than 90% of the CWs systems implemented in Portugal are horizontal subsurface flow. The 5 most used plants in pilot studies and in real CW systems are *P.australis*, *T. latifolia, A. donax, I. pseudacorus* and *C. inidica*. Most of the plants are native, with the exception of *A. donax* that despite being invasive, once introduced and abundant in the region around the systems, showed good performance in pollutant removal and survivability.
- Most of the studies were carried out in the treatment of both industrial and domestic wastewater, which has been the major focus of research in CWs. Within the industrial context, as Portugal is a country where agriculture is an activity of great economic importance, more studies relating the potential of CWs systems in the rural sector may be an area of interest in the future, since it brings the possibility of testing more sustainable agricultural systems, allowing the reuse of wastewater and the valorization of biomass in internal processes.
- Regarding the substrate, the expanded clays showed excellent performance to treat wastewater contaminated with metals and other pollutants from industry as well as organic matter, pharmaceuticals and other pollutants from domestic wastewater. The possibility of exploring sustainable substrates such as cork, brick waste among others, increasing their useful lifetime was also presented in this research as it has already been explored in some cases in Portugal, as the case of the wine industry.
- Regarding to the real scale systems, the majority part of CWs were performing treatment for WWTPs. Of the 37 WWTPs found, only 5 responded to the contacts stating that the systems were still working properly and functioning adequately. Of the industrial systems, the most studied industry was the tannery industry, having over 20 years of research using CW with positive results. A CW in a tourism unit - Paço de Calheiros - was the system that covered most different studies in this area. Besides being the pioneer in testing an ornamental polyculture as vegetation, multidisciplinary studies were and are being carried out in this system, being of great relevance to the academic community and to the society.
- Regarding FWIs systems, the systems found were exclusively made to phytoremediate port wastewater. The most used types of platforms were made of cork, a material that comes from a native tree (*Quercus suber*) in Portugal. The most used plants were *S. perennis*, *H. portulacoides*, *I. crithmoides*, and *S. maritima*. Of these, *S. perennis* was the most survivable plant followed by *S. maritima*. There are indications that biofouling could affect the FWI buoyancy, although more studies are needed.

- Ecosystem services were provided by the systems studied. Regarding the CW systems, the results pointed directly to functions related to ornamental resources, water purification, biodiversity enrichment, nutrient cycling, and cultural (tourism and aesthetic). However, indirectly it is possible to assume that once implemented, the real systems also provide air purification and microclimatic effects, and support biodiversity (pollination and presence of microorganisms mainly). Studies that aimed to reuse the treated water for irrigation, evaluate the increase of bird biodiversity, or reuse/valorize the generated biomass were not explored, being interesting points to be explored in the future.
- FWI's can provide services such as biomass for energy as biomass to produce bioenergy such biofuel, carbon sequestration, nutrient cycling and uptake, water cleaning, carbon sequestration, aesthetic values and integration, biodiversity support and educational purposes, research and opportunity for training. The ecosystem services provided by the systems studied focused mainly on the recycling and uptake of nutrients and pollutants, biodiversity support since this service was observed as a consequence of the implementation of the islands, including the one implemented as part of this study, and aesthetics. Benefits such as carbon sequestration and provision of valuable biomass have not been studied, a gap to be filled in future studies. Investing in the possibility of integrating FWI educational in blue carbon research could be a good suggestion for future studies.

5. Publications in scientific meetings

Oliveira L. W., Vieira N., Calheiros C. S. C. 2021. Wastewater management in Portugal through phytoremediation. In: Livro de Resumos do 14.º Encontro de Jovens Investigadores da U.PORTO. ISBN 978-989-746-295-5 (resumo)

Oliveira L. W., Vieira N., Calheiros C. S. C. 2021. Wastewater management in Portugal through phytoremediation. 14.º Encontro de Jovens Investigadores da U.PORTO. 0507/05/2021, Universidade do Porto (poster)

Oliveira L. W., Vieira N., Calheiros C. S. C. 2020. Phytoremediation in Portugal: application of plants to treat different wastewaters. In: Book of abstracts: 4th Meeting – online edition – Functional Biology and Biotechnology of Plants. 2 december 2020. Published by: Biology department of the Faculty of Sciences – University of Porto, Portugal. (pp39).

Oliveira L. W., Vieira N., Calheiros C. S. C. 2020. Phytoremediation in Portugal: application of plants to treat different wastewaters. 4th Meeting – online edition – Functional Biology and Biotechnology of Plants. 02/12/2020. Biology department of the Faculty of Sciences – University of Porto, Porto, Portugal. (Poster)

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Annex 1

Table 18: Repositories of the Portuguese universities surveyed.

University	Repository source
Universidade de Coimbra	https://www.uc.pt/fctuc/BibliotecasFCTUC/bibl_poloII/pesq_rec_eletr/repositorios
Universidade dos Açores	https://repositorio.uac.pt/
Universidade do Minho	http://repositorium.sdum.uminho.pt/handle/1822/611
Universidade de Algarve	https://sapientia.ualg.pt/?locale=en
Universidade de Evora	http://dspace.uevora.pt/rdpc/
Universidade de Trás-os-Montes e Alto Douro	https://www.rcaap.pt/repositoryInfo.jsp?id=utad
Universidade nova de Lisboa	https://run.unl.pt/
Universidade Fernando Pessoa	https://bdigital.ufp.pt/
Universidade de Aveiro	https://ria.ua.pt/
Universidade Católica Portuguesa	https://repositorio.ucp.pt/
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Universidade de Lisboa	https://repositorio.ul.pt/
Universidade técnica de Lisboa	https://www.repository.utl.pt/
Universidade de Maia	https://repositorio.ismai.pt/
Universidade autonoma de Lisboa	https://repositorio.ual.pt/
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Universidade Lusiada	http://repositorio.ulusiada.pt/
Universidade Europeia Lisboa	https://comum.rcaap.pt/handle/10400.26/6753
Atlântica instituto universitário	https://repositorio-cientifico.uatlantica.pt/
Universidade Aberta	https://repositorioaberto.uab.pt/
Instituto Superior Miguel Torga	https://repositorio.ismt.pt/
Universidade Lusófona de Humanidades e Tecnologias	https://recil.grupolusofona.pt
Universidade Portucalense	http://repositorio.uportu.pt/

Annex 2

Email/ questionnaire sent to the wastewater treatment plants in Portugal that based on the literature, applied Constructed Wetlands systems. Original version and English translation are showed bellow.

“Prezados,

Meu nome é Letícia Weber e estou escrevendo minha tese de mestrado sobre o tema fitorremediação em Portugal. Com o intuito de completar minhas pesquisas bibliográficas gostaria de confirmar as seguintes informações a respeito da FITOETAR que foi implementada em xxxx. Também agradeceria se pudessem me enviar as perguntas respondidas a respeito de outras FITOETAR (caso exista) que esteja sob sua supervisão.

1- *Esta FITOETAR ainda se encontra em funcionamento?*
2- *Caso SIM, teria como me enviar os dados da última análise físico-química? Todas as plantas ainda estão vivas desde que o sistema foi implementado?*
3- *Caso NÃO, qual foi o motivo de não ter dado certo?*

Melhores cumprimentos,
Letícia.”

"Dear,

My name is Letícia Weber and I am writing my master thesis on the subject of phytoremediation in Portugal. In order to complete my bibliographical research, I would like to confirm the following information about the constructed wetland (CW) system that was implemented in xxxx. I would also be grateful if you could send me the answered questions concerning other CW (if any) under your command.

1- Is this CW still in operation?
2- If YES, could you send me the data of the last physical-chemical analysis? Are all the plants still alive since the system was implemented?
3- If NO, what was the reason why it did not work out?

Best regards,
Leticia."

Annex 3

Table 19: Constructed Wetlands real scale systems in Portugal

System designation	Type of flow			Treatment stage		Scale			Substrate			References (*)
	Subsurface		Superficial									
	SSV	SSH		2º treatment	3º treatment	Industrial	Community	Residential	Expanded clay	Sand	Gravel	
Leather company North Portugal		1		1		1			1			27, 28, 29, 30, 36, 37
Leather company center Portugal		1			1	1			1	1		31, 34, 35, 36, 37
Small community domestic North PT		1			1			1			1	140
Tourism unit at Paço de Calheiros		1		1				1	1			41, 43, 44, 45, 46, 48, 42, 40, 33
System of Barroca d' Alva – Alcochete (SAlc.)		1		1			1				1	86, 85, 188, 18, 137
System of Aranhas – Penamacor		1		1			1					86, 85, 188

System of Casal de Frade – Arganil		1		1			1					86, 85, 188
System of Póvoa das Forcadas – Carregal do Sal		1		1			1					86, 85, 188
System of Anobra – Condeixa(Scondx.)		1		1			1					86, 85, 188
System of Erada – Covilhã		1		1			1			1	1	86, 85, 188
System of Lousal – Grândola		1		1			1					86, 85, 188
System of Gonçalo – Guarda (SG)		1		1			1					86, 85, 188
System of boa ventura - Madeira (SM)		1		1			1					86, 85, 188
System of Grada – Mealhada Utad1		1		1			1					86, 85, 188
System of Malavado – Odemira (SM)		1		1			1				1	86, 99
System of Rossas – Vieira do Minho(SR)		1		1			1			1		86, 85, 188
System of Salamonde 1 – Vieira do Minho		1		1			1			1		86, 85, 188

System of Salamonde 2 – Vieira do Minho (SS2)		1		1			1			1		86, 85, 188
System of Salvador – Penamacor (Ssalv.)		1		1			1					86, 85, 188
System of Castelejo – Santa Comba Dão		1		1			1					86, 85, 188
System of Adiça – Tondela		1		1			1					86, 85, 188
System of Vale – Tondela		1		1			1					86, 85, 188
System of Penedo – Vila de Rei (SVR)		1			1		1					86, 85, 188
Travanca do Mondego		1		1			1					99
Porto da Raiva		1		1			1					99
Oliveira Mondego		1		1			1					99
Coiço		1		1			1					99
Cunhedo		1		1			1					99
Silveirinho		1		1			1					99
Cruz do soito		1		1			1					99
S.Paio Mondego		1		1			1					99

Meimão		1		1			1					99
Amiais		1		1			1					99
Galifonge		1		1			1					99
Lustosa		1		1			1					99
Ribafeita		1		1			1					99
Criação		1		1			1					99
System of S. Pedro d'Alva – Penacova (SPNC)		1		1			1				1	188
System of Sarnadas Rodão		1		1			1			1		183
Small community domestic North PT 2		1		1				1				140
System of Fataca(SF)		1									1	100
TOTAL		41	0	37	3	2	35	3	3	6	6	

(*) The numbers represent the numbering of bibliographic references.

SSV= subsurface vertical flow; SSH = subsurface horizontal flow