CONSTITUENTS OF THE CONSTRUCTED WETLANDS WITH VERTICAL SUBSURFACE FLOW FOR WASTEWATER TREATMENT: A REVIEW

Abstract
In this work, it is presented a summary of research articles on the main constituents of the constructed wetlands with vertical subsurface flow as well as the state of the art of this technology. Data collection was carried out at the databases Science Direct® and Springer© and the words vertical, up flow, constructed wetland, treatment, macrophyte and substrate were used. In the first stage, they were found 164 articles and, among these, only 42 had reported aspects about the objective of this study. The results indicated that nearby 66% of wetlands constructed with vertical flow are on a pilot scale. In addition, 100% of the systems used emergent macrophytes, with the emerging species Phragmites australis (Cav.) Steud. (PH) in 21% of the wetlands. Conventional materials such as sand and gravel are still used as substrate in most systems (59%). It was also observed that 59% of the wetlands were used for domestic and/or municipal wastewater treatment and that the Asian continent had the largest number of publications of this type of system, with 50% of articles with Asian origin. The constructed wetlands with vertical subsurface flow stood out in nitrogen removal because of the depletion of oxygen in the medium existing in the base of the unit, creating anaerobic / anoxic conditions. In this way, the right choice of the constituents of this system presents an important alternative for the treatment of wastewater.

Keywords: ecotechnology, macrophytes, microorganisms, substrates, phytoremediation.

1 Postgraduate Program in Ecology and Natural Resources, Federal University of Ceará, Brazil. 2 Department of Chemistry and Environment, Instituto Federal do Ceará, Brazil.

* Corresponding author: Department of in ecology and natural resources, Building 906, Federal University of Ceará, 34 UFC, Zip Code: 60440-900, Fortaleza, CE, Brazil. Email: vmsbermudez@gmail.com
Introduction

Water is a natural resource of vital importance for the development of any country and, for this reason, one of the 17 UN Sustainable Development Goals (SDG) is dedicated to water and sanitation. In this way, there is a concern to ensure the availability and management of water and sanitation to all in a sustainable way. However, the increasing demand for water consumption (2030 WRG, 2009; WWAP, 2012) and the lack of basic sanitation generate impacts to the ecosystem, biodiversity and sustainability.

According to Almuktar et al. (2017), contamination of the environment in several ways has restricted access to water and generated ecosystemic, economic and social imbalance, rendering water sources inappropriate for irrigation and drinking. Besides, other negative impact factors on water availability are population growth, global warming and climate change.

Wastewater can be defined as water from polluting sources such as domestic, agricultural and industrial, and its composition varies according to its origin (Li et al., 2017). Thus, due to this variety of composition, reuse of effluents should be monitored for the protection of water bodies and the safety of reuse activity. In this context, we highlight the research for treatment systems with efficiency, reliability, low operational cost and deployment that occupies little space (Von Sperling, 2014). Constructed wetlands (CW) are a wastewater treatment alternative with operating characteristics that set them apart from conventional systems. Those systems are an ecotechnology that aims to minimize damage to ecosystems and, at the same time, to promote sustainability (Mthembu et al., 2013).

According to Vymazal and Kropfelová (2008), CW can be classified in surface flow and subsurface flow systems, which are divided into horizontal and vertical. The choice of the type of system depends on the effluent to be treated. The CW is based on the principles of natural wetlands that occur in the ecosystem and reproduce the hydrodynamic mechanisms, but with greater control of the hydraulic system (Vymazal, 2007). They are basically constituted by macrophytes, substrate and medium microbiota. Because of this, CW results in a cost-effective and versatile treatment system (Ilyas; Masih, 2017).

The substrate supports the macrophyte and microorganisms, and functions as an electrical conductor. Microorganisms are responsible for the removal of pollutants and organic matter in symbiosis with macrophytes (aquatic plants). When the size of the substrate is small, the surface availability is higher, which contributes to the growth of biofilms and increased concentration of microbial cells that form the system microbiota (Scholz; Xu, 2002). The bacteria present in the roots of aquatic plants receive oxygen from the plants and, in turn, perform the decomposition of organic matter present in wastewater converting it into nutrients necessary for macrophyte development (Kadlec; Knight, 1996).
Environmental and operational issues related to the use of CW are generally reported in review articles (Bi et al., 2019; Ingrao; Failla; Arcidiacono, 2020). However, there are factors that influence the performance of the wetland and that are not commonly mentioned in the literature, which makes it difficult to standardize its dimensioning. These factors are such as the type of residual water to be treated, the volumetric organic load (Bakhshoredeh et al., 2020; Liu et al., 2019), the substrates used as fillers (Mlih et al., 2020; Wang; Xu; Sheng, 2020) and the system configuration (Ji et al., 2020; Rous, Vymazal; Hnátková, 2019).

These reviews are important as they are quick tools for the dissemination and contextualization of knowledge. Although many advances have been made, the technology still lacks a more specific review of vertical wetlands as these systems are the most recommended for removing nitrogen and suspended solids and can operate with intermittent feeding in short periods in order to prevent clogging and improve the aerobic conditions of the system (Vymazal, 2007).

Therefore, this review will address the characteristics of the main constituents of the constructed wetlands with vertical subsurface flow (CWVF) and their innovations. It is expected to provide a critical assessment capable of improving treatment efficiency in future studies and applications.

Procedure for data collection
A literature search was performed on the main constituents of the CW with vertical subsurface flow – macrophytes, substrates, hydraulic retention time (HRT), system size, microbial community – and the configurations required for wastewater treatment.

Data collection was performed in April 2019 and two scientific manuscript search websites were used as databases, Science Direct® and Springer© Link. It was defined as inclusion criteria only research articles published between 2016 and 2019 to obtain the current state of the technology.

The following keywords were used: up flow, constructed wetland, treatment, macrophyte and substrate. After the inclusion criteria and the use of keywords, abstracts were read as exclusion criteria of articles and those that had no vertical flow wetlands approach were excluded. Finally, the articles were completely read and the necessary data extracted and these data were summarized and interpreted.

Results and discussion
From a total of 164 articles found in the initial search, 42 were selected for reading and data analysis. All selected articles had English as their writing language, which is the predominant language used in manuscripts published worldwide for most areas, according to Jirge et al. (2017).
The year of 2017 concentrated the largest number of articles published, followed by the year of 2018, with 14 and 12 articles, respectively. However, the year of 2019 already had 11 publications in the first half of the year, when the search was performed. The year of 2016 had only 5 publications. It is illustrated in Figure 1.

Figure 1. Number of publications during the years of 2016, 2017, 2018 and 2019.

Regarding the place of origin, 50% of the publications had Asian origin (Figure 2), with the largest representative being China and India, with 24% and 14%, respectively. Both countries are the most populous in Asia. In central Asia, there is a predominance of arid and semi-arid climates and, in addition to increasing demand for water, the region faces difficulties with water quality due to overexploitation of groundwater, marine invasion, depletion and salinization of water aquifers (Tripathi, 2011).

Immediately thereafter, Europe appears with 31% of the manuscripts, especially the United Kingdom, which is the continent's largest representative in technology publications (9%). According to data from the European Environment Agency (EEA, 2018), 80% of the water resources used in Europe were from inland surface waters and groundwater, sources that are seriously vulnerable to climate change. About 40% of this water is used in agriculture. The EEA also estimates that approximately one third of the territory of the European Union is permanently or temporarily exposed to water scarcity conditions. Thus, population
growth, urbanization, pollution and water scarcity have led to Asian and Europe having the largest percentage of research articles on constructed wetlands with vertical subsurface flow, a simple and inexpensive alternative for wastewater treatment (Rashid; Manzoor; Mukhtar, 2018).

**Figure 2.** Percentage of research articles on vertical flow CW as a function of the continent of origin.

*Constructed wetlands with vertical subsurface flow (CWVF)*

The CWVF system consists of a flat bed filled with substrates where macrophytes are planted. The wastewater is gradually added until the surface floods and percolates all over the bed to the base of the unit, and then collected by a drainage system (Vymazal; Kropfelová, 2008), as shown in Figure 3. Von Sperling and Sezerino (2018) emphasize the importance of choosing system configuration. These authors point out that factors such as construction cost, sustainability, operating costs and simplicity should be taken into consideration.

An important variable for the correct dimensioning of the system is the organic load rate (OLR) as it allows it to operate with greater efficiency (Metcalf; Eddy, 1991). It can be obtained from the flow product by the concentration of organic matter to be chosen. Only 23% of the selected articles reported the value or contained data to calculate the OLR of the system, as shown in Table 1.
Table 1. Overview of organic loading rate in CWVF.

| Authors           | Organic loading rate (Olr) | Effluent                                |
|-------------------|---------------------------|-----------------------------------------|
| Kraiem et al. (2019) | 0.062 BOD₅ kg.day⁻¹       | Rural wastewater                        |
| Chen et al. (2019)   | 9.75 BOD₅ kg.day⁻¹        | Domestic and rural wastewater           |
| Stefanakis et al. (2019) | 473.73 BOD₅ kg.day⁻¹     | Municipal sewage                        |
| Silvestrini et al. (2019) | 2.34 BOD₅ kg.day⁻¹       | Diluted landfill leachate               |
| Verma; Suthar (2018) | 0.023 BOD₅ kg.day⁻¹      | Dairy wastewater                        |
| Hijosa et al. (2016)   | 0.2592 BOD₅ kg.day⁻¹      | Urban wastewater                        |
| Ma et al. (2017)       | 0.039 BOD₅ kg.day⁻¹      | Synthetic river water polluted with cadmium |

*BOD₅: Biochemical oxygen demand.*

It was observed in Table 1 that the authors Stefanakis *et al.* (2019) stood out for having a high OLR (473.73 BOD₅ kg.day⁻¹). This is due to the type of affluent that was treated (municipal sewage) which influenced the size of the CWVF they operated (1160 m²). In contrast, the authors Verma and Suthar (2018) with OLR of 0.023 BOD₅ kg.day⁻¹ operated on CWVF of the 2.75 m², so that the greater the organic load applied, the greater the area requirement. This type of technology becomes very attractive considering the treatment of domestic sewage, particularly in rural areas.

When using rural wastewater as an affluent, the authors Chen *et al.* (2019) and Kraiem *et al.* (2019) obtained different organic load rates. This is because the affluents of the authors Chen *et al.* (2019) were domestic and rural wastewater with hormones and biocides from livestock. Therefore, the organic matter present in this rural wastewater is greater than that of the authors Kraiem *et al.* (2019), whose source of affluent did not use pollutants with a high organic load. Both treatments obtained BDO removal of above 80%.
Concerning the CWVF, it can be mounted on both the laboratory scale (microcosm scale) and the pilot scale, and of the total of selected articles, 33% were performed on the microcosm scale.

The microcosm CWVF is performed in environments with limited space available for full-scale construction and surface area of less than 0.5 m² (Li et al. 2014). The mean dimensions observed for the systems presented in the reviewed articles were $0.42 \pm 0.16$ cm in length, $0.28 \pm 0.05$ cm in width and $0.49 \pm 0.20$ cm in height. The dimensions commonly used in different works are presented in Table 2.

| Authors                  | Length (Cm) | Width (Cm) | Height (Cm) | Macrophytes                                      |
|--------------------------|-------------|------------|-------------|--------------------------------------------------|
| Hu et al. (2018)         | 0.30        | 0.30       | 0.50        | Phragmites australis                             |
| Ma et al. (2017)         | 0.25        | 0.36       | 0.50        | Iris sibirica                                    |
| Hehman et al. (2017)     | -           | -          | 0.80        | Typha latifolia and Phragmites australis          |
| Das et al. (2019)        | 0.63        | 0.17       | 0.62        | Canana India                                     |
| Ramirez-Vargas et al. (2019) | -          | -          | 0.5         | Juncus effusus                                   |
| Santos et al. (2019)     | 0.40        | 0.30       | 0.30        | Phragmites australis                             |
| Perez-López et al. (2018) | -          | -          | 0.40        | choenoplectus americanus                         |
| Tunker et al. (2017)     | 0.65        | 0.30       | -           | Typha latifolia                                  |
| Almeida et al. (2017)    | 0.40        | 0.30       | 0.30        | Phragmites australis                             |
| Samal et al. (2017)      | -           | -          | 0.90        | Canna indica                                     |
| Silvestrini et al. (2019) | 0.25       | 0.25       | 0.35        | Typha domingensis, Scirpus californicus, and Iris pseudacorus |
| Abdelhakeem et al. (2016) | 0.30       | 0.30       | 0.30        | Phragmites australis                             |
| Yan et al. (2016)        | 0.60        | -          | 0.35        | Vetiveria zizanioides                            |
| Ping et al. (2018)       | -           | -          | 0.70        | Iris pseudacorus L.                              |

(-) Value no information

In addition to the reduced space, according to Almeida et al. (2017), studies with CWVF in microcosm scale still have the advantage of allowing greater control of experimental conditions. This is important for understanding and improving technology. These authors studied the treatment of wastewater from agricultural processes contaminated with antibiotic commonly...
used in Portuguese livestock (enrofloxacina or ceftiofur). A microcosm scale CWVF was used to fully control the experimental conditions in order to obtain an efficient delineation of the optimal conditions for the process.

The same justification was used by Ma et al. (2017), who operated a microcosm-scale CWVF in the most wired months in China (September to December) to remove cadmium in a synthetic effluent simulating contaminated river water. Four pollutant concentrations (1, 2, 4 and 8 g.L\(^{-1}\)) were studied. This system had an area of 0.18 m\(^2\), was gravel filled and planted with *Iris sibirica* Linnaeus, which was chosen because it is resistant to low temperatures and due to the smaller scale of the system, that allows with greater precision the observation of macrophyte performance, pollutant removal mechanisms and microbial enzymatic activity.

A microcosm-scale CWVF, projecting environmental conditions from a wireless monitoring network for domestic wastewater treatment was projected by Hehman et al. (2017). The system used the macrophytes *Typha latifolia* Linnaeus and *Phragmites australis* Trinius and was incubated in a growth chamber to test different optimum temperatures using an automatic heater and different light intensities using fluorescent lamps, besides the influence of these factors on the release of dissolved oxygen (DO) through the macrophyte rhizosphere to improve the pollutant removal efficiency. The system was made with plastic containers with 44 cm in diameter and filled with gravel of varying sizes (20 to 30 cm). The microcosm scale allowed them to evaluate the best ecosystemic condition for each macrophyte. For *T. latifolia* L., 30°C to 35°C and 15.000 lx, and for *P. australis* (Cav.) Steud. (PH), 35°C and 10.000 to 15.000 lx, were the optimum temperature and light intensity, respectively, for the highest DO release. Therefore, under these conditions, the best pollutant removal efficiency was favored.

Butterworth et al. (2016) studied the effect of artificial aeration on CWVF on a pilot scale, which had 4 tanks (56 m x 12.5 m), one with *T. latifolia* L., the other with *P. australis* (Cav.) Steud. (PH), both with artificial aeration and the other 2 without artificial aeration, only with the corresponding macrophytes as process control. *T. latifolia* growth rate was higher than *P. australis* (Cav.) Steud. (PH), but when compared to its non-aerated controls, *T. latifolia* L. had higher negative visual effect (yellow leaves and stunted growth). The authors justified that impacts may be species-specific, for example on *P. australis* (Cav.) Steud. (PH) they were less intense.

Therefore, the source and flow of wastewater to be treated define the wetland modality to its configurations and its dimensions. In addition, it is also important to assess the climate of the region where the system will be installed, as it has an impact on metabolism in the dynamics of the organisms that perform the treatment (Moomaw et al., 2018).
Although they are easy to handle, periodic maintenance is necessary so that they do not compromise the efficiency of the system and hydraulic anomalies occur, such as the engagement of pipes and the removal of sludge that is formed in the system, which increase the suspended value in the system.

**Types of wastewaters treated in CWVF and nitrogen removal mechanism**

Wastewater can be divided into 5 types: municipal or domestic, industrial, agricultural wastewater, runoff water and landfill leaching (Vymazal;Kropfelová 2008). Among the reviewed articles, in 59% of them, the CWVF system treated municipal or domestic wastewater, 26% was fed with industrial, 8% with agricultural wastewater, 5% with landfill leachate and 2% with runoff water (Figure 4).

![Figure 4. Wastewater types used for CWVF treatment.](image)

Nitrogen is one of the most important pollutants in wastewater and when in excess causes a great impact due to the problem of eutrophication of aquatic environments (He; Xue; Wang, 2009; Von Sperling, 2007; Zoppas *et al.*, 2016). The main nitrogen conversion pathways that occur in wetlands systems, such as ammonification, nitrification, denitrification, biological fixation, nitrate ammonification, ammonia anaerobic oxidation (ANAMMOX) and volatilization, are presented in Figure 5.

Ammonium can also be partially converted to nitrite by the CANON process (“Completely Autotrophic Nitrogen removal Over Nitrite”), in which the aerobic oxidizing ammonia perform the conversion (Jetten *et al.*, 2002), and later the ANAMMOX bacteria convert the nitrite produced and part of the remaining ammonium to gaseous $N_2$, and nitrate is formed in small amounts.
Considering that possible damage to the environment may occur due to nitrogen transformations, some researchers have been studied processes mediated by microorganisms. According to Silveira et al. (2015) and Wu et al. (2015), CWVF is the most suitable for nitrogen removal because of the existence of anaerobic/anoxic conditions at the base of the system. It was observed in the upper part of the unit that aerobic conditions favor the oxidation of organic matter and nitrification, and the anaerobic/anoxic conditions existing in the base favor the reduction of organic matter and denitrification (Chen et al., 2015).

Nitrification reactions, with the oxidation of ammonia to nitrite and nitrite to nitrate, are mediated by nitrifying bacteria that obtain energy to grow from the oxidation of inorganic nitrogen compounds (Lee; Fletcher; Sun, 2009). Thus, dissolved oxygen is an important factor for the nitrification process to occur, but at lower concentrations it may limit the activities of these microorganisms. When in high concentrations it has high oxidizing power. It is also a limiting factor for self-purification measured from the oxidation-reduction potential (ORP).
On the other hand, the denitrification occurs in anoxic zone by action of heterotrophic bacteria in which nitrate is reduced to molecular nitrogen (Hidaka et al., 2002). Nitrogen is also present in constructed wetlands in the organic form of urea, amino acids, amines, pyrimidines and purines (Vymazal, 1995).

Microbial nitrification followed by denitrification are known as the main pathways for nitrogen removal from the environment, and available oxygen is a key factor for the growth of nitrifying bacteria (Hidaka et al., 2002).

Some alternative ways for nitrogen removal have been studied in CWVF systems, such as Anaerobic Ammonia Oxidation, known as ANAMMOX, which consists of anaerobic conversion of NO₂ and NH₄⁺ to N₂ (Van de Graaf et al., 1995). Kraiem et al. (2019) investigated ANAMMOX bacteria for 4 months in two CWVF systems and one up and horizontal flow hybrid system (UHFHS) to treat rural wastewater, comparing two abundant macrophyte species from Tunisia, Phragmites australis (Cav) Steud. (PH) and Typha angustifolia L. (TA). In the hybrid system, PH was planted in the vertical flow cell and TA was planted in the horizontal flow cell. In the individual CWVF systems, each one received a single macrophyte species to evaluate the role of the plant in the process, comparing individual and hybrid systems. The authors observed that the three systems presented high efficiency in the removal of chemical oxygen and nitrogen demand. However, the hybrid system achieved the highest Kjeldahl total nitrogen removal, which was justified by the fact that it has two different flow configurations, which established favorable conditions for removal. Therefore, it was beneficial to use a horizontal flow cell after a vertical flow cell, resulting in better wetland system performance. T angustifolia L. (TA) stood out for favoring the growth of ANAMMOX bacteria even in co-existence with heterotrophic bacteria, indicating that its application in CWVF was the best setting to promote ANAMMOX activity. The authors additionally said that the ANAMMOX process did not require organic carbon and reduced the production of greenhouse gases, unlike the conventional denitrification process.

The nitrogen removal from wastewater and the use of aeration to create an environment with alternating aerobic and anaerobic zones and, thus, favor pollutant removal in a CWVF was an efficient system studied by Al-isawi et al. (2017) and Jia et al. (2018). Jia et al. (2018) used intermittent aeration in a CWVF system to treat domestic wastewater. The use of intermittent aeration in CWVF systems resulted in better nitrogen removal with 99% ammonia and 96% total nitrogen, which was also ratified Dong et al. (2012). These authors, by promoting the addition of oxygen to the system, recorded a total nitrogen removal of more than 57%.
In other research, a comparison was made between wetland and artificial pond systems for domestic wastewater treatment. Thus, Al-Isawi et al. (2017) studied the influence of aeration on CWVF and simultaneously on three artificial pond types for five years. The first system contained wastewater only, the second consisted in wastewater and \textit{P. australis} (common reeds) and the last one was composed by residual water, macrophyte and aeration addition. The artificial ponds were made with cylindrical plastic buckets, in which they were placed in a large concrete container filled with soil to mimic the natural conditions of a pond. The authors observed that the aerated lagoon presented better results than the CWVF that had no artificial aeration system in relation to ammonia removal. Therefore, the results corroborate the authors Kadlec and Wallace, 2009 and Scholz, 2015, who stated that aeration is important during the ammonia reduction procedure. According to Yoo et al. (1999), the way of aeration is also important, since when it was intermittently used in short cycles, it can allow nitrification and denitrification to occur at the same time.

Aeration also influences the greater removal of indicators of fecal contamination. Stefanakis et al. (2019) observed strong correlations between bacteria and bacteriophages present in domestic effluent treatment in a biological sedimentation tank system and in aerated CWVF. Bacteriophages acted as bacterial predators and were still used as indicators of microbial removal in both evaluated treatment systems. Besides, the aerated CWVF had higher microbial contamination removal efficiency.

It is also important to report that wetland systems were efficient not only in the treatment of discharges with easily biodegradable organic matter and nutrients, but have been used to remove compounds hard to biodegrade such as those found in industrial wastewater, as shown in Table 3.

Industrial wastewater has the greatest environmental impact and, in most cases, its toxicity is associated with the presence of compounds that are difficult to remove (Okereke;Ogidi;Obasi, 2016). Yan et al. (2016) used a CWVF to treat synthetic wastewater from the drug industry. In their system, it was used the macrophyte \textit{Cyperus alternifolius} Willdenow with gravel and sand as the substrate employed. The performance of the system on the effect of four selected pharmaceutically active compounds (PhACs) – carbamazepine (CBZ), sulfamethoxazole (SMZ), ofloxacin (OFX) and roxithromycin (ROX) – on photosynthesis and enzymes released by the macrophyte was evaluated, as well as the pollutant removal efficiency. The pollutant removal efficiency was found to be equal to or even better than that achieved in conventional water treatment systems (decantation, filtration, coagulation, flotation, activated sludge, biofilters), getting 90% of OFX removal, 62% of CBZ, 84% of ROX and 78% of SMZ. They also evaluated that the emerging macrophyte \textit{C. alternifolius} was able to absorb and support part of the PhACs (pharmaceutically active compounds), keeping their photosynthetic activity and enzymatic defense active. The treatment and supervision of this effluent is extremely important, because when it is not properly released it pollutes the bodies of water and causes toxic effects on the microorganisms of the environment (Jin et al, 2017).
**Table 3. Overview of industrial wastewater types.**

| Industry       | Pollutant | (C₀ - Cᵢ) Efficiency (%) | Authors                                      |
|----------------|-----------|---------------------------|----------------------------------------------|
| **Food**       | Diry      | 70%                       | Sharma et al. (2018)                         |
| **Textile**    | Dye       | 80%                       | Chandanshive et al. (2017)                   |
| **Chemical**   | Sanitizing Detergents | - 90%                   | Peréz-lópes; Arreola-Ortíz e Malagon-Zamorra (2018) |
| **Chemical**   | Oil Refinery | 76%                       | Mustapha; Bruggen e Lens (2018)              |
| **Pharmaceutical Medicines** | Nanosilver | 96%                       | Huang et al. (2018)                          |
|                | Ibuprofen  | 10.4 - 0.29 μg.L⁻¹        |                                              |
|                | Ketoprofen | 0.75 - 0.16 μg.L⁻¹        |                                              |
|                |            | -78%                      |                                              |
|                | Naproxen   | 1.74 - 0.47 μg.L⁻¹        |                                              |
|                |            | -73%                      |                                              |
|                | Diclofenac | 0.41 - 0.41 μg.L⁻¹        |                                              |
|                |            | 0%                        |                                              |
|                | salicylic acid | 15.9 - 0.79 μg.L⁻¹       |                                              |
|                |            | -95%                      |                                              |
|                | Caffeine   | 19.2 - 0.26 μg.L⁻¹        |                                              |
|                |            | -98%                      |                                              |
|                | Carbamazepine | 0.99 - 0.41 μg.L⁻¹    |                                              |
|                |            | -58%                      |                                              |

*C₀: affluent concentration; Cᵢ: effluent concentration; NR: no removal; -: Uninformed.*
Other products that are difficult to remove in wastewater are sanitizers (Jensen, 1999). The use of these products means that domestic effluents have certain characteristics of industrial wastewater as there is a wide variety of cleaning products, each of which has a function. Detergents are sanitizers that have surfactants, compounds that break the surface tension of water and thus remove surface dirt (Thomas et al., 2017). According to Giagnorio et al. (2017), effluents with high detergent concentrations, when in contact with the aquatic ecosystem, promote the increase of solids, chlorine and organochlorine concentration, besides the increase of pH. Therefore, an environmental impact is generated on this ecosystem.

With incorrect disposal of polluted effluent in water bodies, fishes are affected by both the pH increase and the presence of solids in the aquatic ecosystem. Calcium oxide can be formed as the pH rises, causing difficulty in gill breathing in fish. On the other hand, solids at higher concentrations may obstruct fish gills, leading to death (Jeziernka; Witeska, 1995). Additionally, pollutants such as organochlorine compounds that are water-soluble can accumulate from the fat tissues of organisms (Kucklick; Baker, 1998), so that contamination of the aquatic environment can often interfere with species morphology and function (animals and plants) in the ecosystem.

Among the reviewed articles, only one performed the treatment of wastewater contaminated with surfactant in CWVF. Located in Durango State, Mexico, it was carried out by Pérez-lópes, Arreola-Ortuñz and Malagon-Zamorra (2018). The CWVF received the macrophyte *Schoenoplectus americanus* (Pers.) Volkart ex Schinz & R.Keller, a native species from Mexico, and were filled with 8 treatment possible with Tezontle (porous volcanic rock), gravel or Agave fiber (plant originated from Central America). Half of these 8 treatments were planted with macrophytes and the other half was not. The graywater from Durang State had hydraulic retention time on 3, 5 and 15 days. However, the better removal of the pollutant surfactants linear alkylbenzene sulfonates (LAS) (90% with 15 days of hydraulic retention time) caused a higher solids generation, low pH and higher toxicity, eliminating the possibility of reuse of this wastewater. In contrast, using only gravel, the removal was 70% and hydraulic retention time of 15 days was recommended for the treatment of greywaters in community.

Water quality in water bodies has also been impaired by improper use of chemicals, including pesticides. These compounds, in general, when in contact with water, cause damage to local fauna, promoting an imbalance of this ecosystem (Agbohessi et al., 2015). Tang et al. (2019) performed the treatment of synthetic wastewater containing the pesticide chlorpyrifos (500 mg.L⁻¹) in CWVF systems. Approximately 98% of pollutant removal was obtained. The system was monitored for 3 months, with 3 operating cycles, each cycle lasting 7 days. The system was 27 cm in diameter and 30 cm high and 5 separate macrophyte species were tested: *Cyperus alternifolius*, *Canna indica* L., *Iris pseudacorus* L., *Juncus effusus* Polish and *Typha orientalis* C. Presl.
CWVF systems have also shown good efficiency in the treatment of landfill leachate, a complex waste in which organic load is difficult to reduce. Silvestrini et al. (2019) used a pilot-scale CWVF in the city of Buenos Aires, Argentina to treat leachate from a 450 ha landfill that produced 600 m$^3$ of raw manure per day. According to the authors, landfill leachate is one of the dumps that presents greater difficulty to be treated by conventional methods. They are discharged with high ammonium concentrations and recalcitrant COD, which are its main characteristics (Wojciechowska et al., 2017). However, the volume and chemical quality of a landfill leachate results from each site, which requires the design and operation of a CWVF to be well planned.

**Macrophytes in CWVF**

According to Cook (1996), macrophyte life forms (growth forms) can be Amphibious, Epiphytes, Rooted emergent, Rooted emergent with leaves and stems Floating, Free Floating, Rooted Submerged and Free Submerged.

Amphibious macrophytes can survive out of water and are also called semi-aquatic. Epiphytes are macrophytes that host other macrophytes. Those that are fully in water are called submerged macrophytes, which can be fixed when they are deep-rooted and free when not deep-rooted, with only flowers emerging out of water. Fixed floats are those that have stem, flower and leaf above the surface of the water and are rooted. There is still the free variation that, by not having rooting, can be carried by the action of the current and float on the surface (Sculthorpe, 1985; Cook, 1996).

Emerging macrophytes are the most abundant in wetlands and swamps. This type of macrophyte grows 0.5 m below the ground surface to a depth of 1.5 m (Wetzel, 2001). According to Westlake (1963), they are the most productive because they have several internal spaces that carry oxygen or their photosynthetic parts are not for roots and rhizomes that are in sub-sediments (saturated) with little oxygen. In these internal spaces are present aeration tissues that are responsible for the support and storage of carbon dioxide and oxygen inside (Sculthorpe, 1985). Among the articles evaluated, 94% of CWVF used emergent-life macrophytes.

The choice of macrophyte to be used in a constructed wetland is extremely important for the success of the system (Brisson; Charazenc, 2009). According to Hoffmann et al. (2011), the choice of species should be based on characteristics such as the ability to withstand periods of water scarcity and to grow in natural wetlands, the type of root system and preference for local species, as shown in Figure 6.
In addition, Moshiri (1993) reported that the species chosen must be tolerant to toxic pollutants that can be present in wastewater. This way, the plant can survive and perform treatment efficiently. In a study on the influence of macrophytes on wetland efficiency, Vymazal (2011) evaluated 35 CW systems and found that the different structures that make up each macrophyte species, as well as their adaptation to the system model used, may favor, hinder or have zero influence on the treatment process.

![Characteristics to consider when choosing macrophyte.](image)

**Figure 6.** Characteristics to consider when choosing macrophyte. *Adapted from Hoffmann et al. (2011).*

According to Hoffmann *et al.* (2011), macrophytes provide a favorable environment for the growth of microorganisms that adhere to their roots and form a biofilm.

Table 4 shows the diversity of species used in CWVF found in this review. All macrophytes used in the reviewed articles belong to the Angiospermae division and the Monocotyledon group. In the Poales order there are the macrophytes most used in CWVF, being the *P. australis* (Poaceae) corresponding to 20.9% of the articles. The Typhaceae family has the second most used species, *T. latifolia* L. and *T. angustifolia* L. (TA) both with 10% of incidence.

The most widely used macrophyte species in the world are *P. australis* (Cav.) Steud. (PH) and *T. latifolia* L. The first is the most popular plant in Europe and Asia, while *T. latifolia* L. is the most
widely used in North America (Vymazal, 2013). According to Luederitz et al. (2001) and Brix and Schierup (1989), these species are the most chosen because they have a deep rhizome, large root system (maintaining electrical conductivity) and because they are present in all regions.

Table 4. Overview of macrophytes used in CWVF

| Division      | Group | Order      | Family       | Specie                      |
|---------------|-------|------------|--------------|-----------------------------|
| Angiospermae  | Monocots | Asparagales | Iridaceae    | Iris pseudacorus L.          |
|               |       |            |              | Iris sibirica L.            |
|               |       |            |              | Spartina patens (Aiton) Muhl.|
|               |       |            |              | Spartina alterniflora Loisel |
|               |       | Poales     | Poaceae      | Phragmites australis (Cav.) Steud. (PH) |
|               |       |            |              | Vetiveria zizanioides Nash   |
|               |       |            |              | Paspalum scrobiculatum L.    |
|               |       | Juncaceae  | Juncus effusus Pollish |
|               |       | Typhaceae  | Typha angustifolia L. (TA) |
|               |       |            | Typha orientalis C. Presl |
|               |       |            | Typha latifolia L |
|               |       |            | Typha domingensis Pers.   |
|               |       | Cyperaceae | Cyperus alternifolius Willd. Ex Kunth. |
|               |       |            | Cyperus papyrus (C.Bauhin ex Kunth) Raf. |
|               |       |            | Schoenoplectus americanos (Pers.) Volkart ex Schinz & R. |
|               |       |            | Scirpus californicus (C.A.Mey.) Sojak. |
|               |       | Pontederiaceae | Pontederia cordata Lour. Fl. Cochin |
|               |       | Zingiberales | Cannaceae. Canna indica L |
|               |       |             |              | Canna glauca L              |

The presence of macrophytes in CWVF is one of the most important constituents for the treatment system. This is because vegetation distributes and reduces water velocity, creating a better condition for sedimentation of suspended solids from the effluent to be treated, which helps to prevent clogging of the system substrate (Somes et al. 1996). In addition, the presence of macrophyte defines the built wetland system as green technology (Stefanakis et al. 2019). Some authors have reported on the importance of observing the macrophyte density factor that would affect the efficiency of wetland systems.

Table 5 presents information on the number of individuals used per m² in wetland systems and species used.
According to Table 5, there is apparently no use of a standard density. However, the range between 20 to 30 individuals per m\(^2\) was the most employed. For example, the species \(T.\ angustifolia\) L (TA), as mentioned earlier, have a deep rhizome and a large root system (Brix;Schierup, 1989), which requires a large amount of space when used in a wetland system. Thus, different densities were recorded for this species: 8, 27, 30 and 50 individuals.m\(^{-2}\) (Al-baldawi, 2018; Hijosa et al. 2016; Turker et al. 2016; Verma; Suthar 2018).

The study of the effects of the number of individuals present in a CWVF on wastewater treatment was not carried out by any article selected for this review, only the presence or absence of macrophytes. Regarding the influence of the type of wastewater under the action of different macrophytes, only 28.5% of the articles selected for this review compared the use of different species of macrophytes submitted to the same effluent.

The removal of Congo red dye and metals from a textile effluent by using the macrophytes \(T.\ angustifolia\) L (TA) and \(P.\ scrobiculatum\) L for 4 days, both in separate cells and in consortia (Chandanshive et al., 2017) resulted in the highest treatment efficiencies achieved when using macrophytes in consortium, being dye removals 94%, COD 70%, BOD 75%, suspended solids - SS
47% and heavy metals - HM 77%. When plants were used in separate cells, *T. angustifolia* L. (TA) had better removal efficiencies - 80% for dye, 65% for COD, 68% for BOD, 35% for SS and 30% for HM - compared to *P. scrobiculatum* L. - 73% for dye, 63% for COD, 63% for BOD, 31% for SS and 28% for HM. Therefore, the use of consortium macrophytes can be a beneficial strategy for the treatment of contaminated wastewater in wetland systems.

On the other hand, the treatment of wastewater contaminated with the pesticide chlorpyrifos in CWVF with different macrophyte species (*Cyperus alternifolius* Willd. Ex Kunth., *Canna indica* L., *Iris pseudacorus* L., *Juncus effusus* Polish and *Typha orientalis* C. Presl. resulted in no significant difference in the pollutant removal values between macrophytes, but there was a substantial increase when compared to the control system (without macrophyte) (Tang *et al.*, 2019) The macrophyte system showed 98% of pollutant removal. The authors also observed that systems containing higher biomass and evapotranspiration macrophytes accelerated the pollutant removal process (*C. indica* L., *C. alternifolius* Willd. Ex Kunth. and *I. Pseudacorus* L.), so that the main pollutant removal pathways were sorption and biodegradation, respectively accounting for 65 to 86% and 8 to 34% of the total removal efficiency.

The effect of the use of macrophytes *C. alternifolius* Willd. Ex Kunth., *I. pseudacorus* L., *C. glauca* L. and *S. validus* (C.A.Mey.) Sojak. on the microbiota present in CWVF was evaluated by Zhang *et al.* (2017). The use of plants in the way of a consortium resulted in greater microbial diversity compared to the situation in which the wetland received only one species or when there was no macrophyte in the system. Diversity occurs as a function of richness and uniformity of the site (Verberk, 2011). Thus, when only species richness was evaluated, that is, only the number of species present in the wetland, the authors observed that the use of macrophytes in consortium or separately did not affect the composition of the communities and concluded that the microbiota can adopt different ecological strategies in response to the presence or absence of macrophytes and richness (Zhang *et al.*, 2017).

In none of the articles analyzed there was a control system (without macrophytes) that achieved better pollutant removal efficiency than those where some macrophyte species was used.

**Substrate in CWVF**

The substrate is the medium used in toilets and are classified into: natural, industrial by-products and artificial products (Meng *et al.*, 2014). It is also called support matrix and filler material, which are important constituents of CWVF for developing important biofilms for microbiota development. But the use of conventional substrates (sand and gravel) faces difficulties in the system, such as low removal efficiency and clogging, so it is necessary to look for substrate alternatives (Zhu *et al.*, 2011).
In order to classify innovative materials recently introduced in wetland systems as substrate, Yang et al. (2018) defined these substrates as emerging, which appear as a new option for greater low-cost treatment efficiency, replacing conventional materials. They classified emerging materials into: ion exchange substrates, sorption substrates, electron donating substrates and others. Among the reviewed articles, 85.7% used conventional substrates. Examples of substrates used in the articles are listed in Table 6.

Table 6. Overview of substrates and hydraulic retention time used in CWVF

| Authors          | Effluent          | HRT (Day) | Substrate and particle size                                                                 | COD (mg.L⁻¹) | NH₄⁺ (mg.L⁻¹) | TN (mg.L⁻¹) | TP (mg.L⁻¹) |
|------------------|-------------------|-----------|----------------------------------------------------------------------------------------------|---------------|---------------|--------------|--------------|
| Jia et al. 2018  | Domestic wastewater | 3         | Gravel (Φ =1-7 cm) + sand (Φ < 1 cm) + wheat straw (NR)                                        | 92.7          | 98.8          | 63           | 52.2         |
|                  |                    |           | Gravel (Φ =1-7 cm) + sand (Φ < 1 cm) + Apricot pit (NR)                                       | 95.3          | 98.4          | 46.2         | 66.7         |
|                  |                    |           | Gravel (Φ =1-7 cm) + sand (Φ < 1 cm) + walnut shell (NR)                                     | 94.9          | 99            | 46.2         | 70.6         |
|                  |                    |           | Gravel (Φ =1-7 cm) + sand (Φ < 1 cm)                                                       | 96.6          | 98.9          | 32.6         | 66.7         |
| Verma and Suthar 2018 | Dairy Industry       | NR       | Sand (NR) + gravel (NR)                                                                    | 83.2          | 66.2          | NR           | 59           |
| Mustafa et al. 2018 | Oil refinery       | 2         | Size grave (Φ = 25 – 36 mm) + small grave (Φ =6-10 cm)                                       | 72.7          | 70            | 68           | 49           |
| Silvestini et al. 2019 | Landfill leachate | 7         | Sand (Φ =0,1 – 0,6 cm) + expanded clay (Φ = 1-2 cm) + gravel (Φ = 2-3 cm)                   | 46            | 66            | 43.6         | NR           |
| Nivala et al. 2019 | Municipal sewage   | 2         | Sand (Φ =1-3 mm) + gravel (Φ =4-8 mm)                                                     | NR            | 69            | 40           | NR           |
| Al-Isawi et al. 2017 | Domestic wastewater | 3         | Gravel (Φ =10mm)                                                                         | 62.8          | NR            | 23.7         | 59.8         |
| Huang et al. 2018 | Residual water     | 1.5       | Gravel (Φ = 1-8 cm + zeolite (Φ = 3-4 cm)                                                | 93.2          | 51            | 60.8         | 65.4         |

COD: Chemical Oxygen Demand; NH₄⁺: Ammonium; TN: Total Nitrogen; TP: Total Phosphorus; HRT: Hydraulic Retention Time; NR: Not Rated; Φ: Diameter.

Hydraulic retention time (HRT) considers the relationship between effluent flow and system volume, as well as the effect of the substrate porosity used, being a variable that indicates the permanence of each fluid component in the system until its exit from it (Grosser, 2017).
According to Table 6, the systems with the lowest HRT provided the largest removals of easily degradable organic matter in terms of COD. Thus, when applying the largest HRT, the authors Silvestini et al. (2019), who used the longest retention time (7 days) among the studies considered, obtained the lowest efficiency of COD removal compared to other authors.

The effectiveness in improving water quality is directly proportional to the retention time of the effluent to be treated within the system (Sezerino and Heleno, 2015), which did not occur when the studies presented in Table 4 were compared.

Pollutant removal in wetland systems can still occur by chemical and/or physical processes such as ion exchange, adsorption, precipitation and complexation (Ge et al., 2015), with the type of substrate being a factor of major relevance to the greater or lesser intensity of these processes. Nevertheless only 38% of the articles evaluated compared the types of substrates for pollutant removal. Among these, Türker et al. (2017) chose 4 substrate types for comparison in a CWVF, with 4 days of HRT and 120 days of wetland monitoring. The substrates used were peat (plant material), zeolite, volcanic ash and sand and the system aimed to remove Boron (B) in drinking water in Turkey. The input of each reactor contained 5–8 mm of gravel at its base. Then there was a single 55 cm deep layer of the main substrate type (peat, zeolite, volcanic ash or sand) and a 5 cm gravel layer was placed at the reactor outlet, resulting in a total depth of 65 cm (main substrate plus gravel layers). Percentages of 91%, 57%, 84% and 83% of pollutant removal were recorded, respectively, for the mentioned materials. This result reinforced what was said by Yang et al. (2018) that the type of substrate influences pollutant removal efficiency.

The treatment of domestic wastewater in CWVF using conventional and emerging substrates was performed with addition of an organic substrate (Table 2). In the case of wheat straw, it promoted an increase in total nitrogen removal when compared to the control (sand plus gravel), with removals of 63% and 36%, respectively (Jia et al., 2018). The very organic matter released by the substrate “wheat straw” was used as carbon source for the present microbiota and, consequently, resulted in greater nitrogen removal.

Therefore, the search for emerging materials that can be used as substrates in CWVF is important for reducing system costs, increasing system efficiency and being able to properly target a material that would no longer be useful.

Regarding the maintenance of the built wetland, the selected articles did not mention the period of cleaning and substrate exchange, unclogging of pipes, checking the reservoir level and macrophyte management. According to the Handbook of Constructed Wetlands (Davis, 1995), produced by the Environmental Protection Agency (EPA), system monitoring should be done to
see if it is achieving good removals of organic matter and other variables and thus identifying and correcting the problem by cleaning the system, changing the substrate or cleaning the pipes.

**Microbial community in CWVF**

According to Ping et al., (2018) it is extremely important to analyze the microbial community in the environment, whether present in the water or in the substrate, as it allows to assess the changes that have occurred. However, only 19% of the articles selected for the review performed the method of DNA / RNA extraction or colony formation unit (CFU) counting to analyze the microbial community in their research, as shown in Table 7.

**Table 7. Overview of microbial community in CWVF**

| Authors          | Affluent                                      | Analysis               |
|------------------|-----------------------------------------------|------------------------|
| Huang et al. (2017) | Synthetic wastewater polluted with nanosilver | DNA/RNA extraction     |
| Kraiem et al. (2019) | Rural wastewater                              |                        |
| Lopardo et al. (2019) | Rural wastewater                              | DNA/RNA extraction     |
| Pelissari et al. (2017) | Domestic wastewater                           |                        |
| Pelissari et al. (2018) | Domestic wastewater                           |                        |
| Ping et al. (2018)    | Domestic wastewater                           |                        |
| Wu et al. (2018)      | Synthetic domestic wastewater                 |                        |
| Ma et al. (2017)      | Synthetic river water polluted with cadmium    | CFU counting           |

The species of the phylum Proteobacteria were the most abundant among the bacteria in the works of Ping et al., (2018), Kraiem et al., (2019) and Lopardo et al., (2019). This abundance is due to the fact that these bacteria participate of biogeochemical cycles (Liu et al., 2016), mainly in the nitrogen cycle. This abundance corroborates with other similar studies (Ansola et al., 2014; Urakawa and Bernhard, 2017, Wang et al., 2017) in which metagenomic analyzes were carried out to study the nitrifying processes in a constructed wetland system.

The diversity and richness of the microbial community was characterized by the authors Ping et al., (2018), Pelissari et al., (2018) and Lopardo et al., (2019) from the Chao 1, Shannon and Simpson indexes. Pelissari et al., (2018) observed that the richness and diversity were greater in the biofilm generated by the constructed wetland than in the analyzed domestic wastewater. Ping et al. (2018) observed that when the system was in hydrodynamic
anomaly, in the case of clogging, the rates decreased, that is, the richness and diversity of bacterial communities were reduced because the production of biofilm was reduced and, thus, the removal of the decaying pollutant (Hua et al., 2010). Therefore, it is possible to say that since the CWVF performance also depends on nitrifying bacteria, the study of the microbial community present in the environment is extremely important.

Conclusion
The constructed wetlands with vertical subsurface flow can have numerous applications and arrangements and can be used for the treatment of wastewater from domestic, industrial leachate and even landfills, among others. So, it is a promising technology for wastewater treatment. However, even though the built wet areas have been studied extensively, it is of great importance to choose the appropriate characteristics of the system components to obtain maximum pollutant removal.

The selection of macrophytes and their characteristics must be carefully studied, since it has a positive impact in the removal of pollutants. For the proper dimensioning of the system, the flow to be used, the hydraulic retention time, the organic load rate and the porosity of the substrate must be considered and macrophyte density per superficial area. It is important to standardize these criteria, since there is no uniformity in the design procedures.

The research for emerging substrates is interesting, as they are generally materials that, after use, lose value, so that their use as a substrate in wet areas minimizes their disposal in the environment, such as construction waste. The study of the microbial community of the environment is important because they play a fundamental role in the removal of polluting organic matter and nitrogen, which, in high concentrations in the effluent to be released, unbalances the ecosystem. Furthermore, they are performance indicators of the system.

However, to ensure the sustainability and useful life of these systems, it is important to carry out periodic maintenance of their constituents, which is a viable activity, considering that they have low cost and are easy to replace and manage.

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