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Use of tropical macrophytes in wastewater treatment

Lara-Acosta, Marimar[1]; **Lango-Reynoso, Fabiola**[1*]; **Castañeda-Chávez María del Refugio**[1]

[1] Tecnológico Nacional de México/Instituto Tecnológico de Boca del Río, Carretera Veracruz-Córdoba km 12, Boca del Río, Veracruz, México, C. P. 94290.
* Correspondence: fabiolalango@bdelrio.tecnm.mx

ABSTRACT

Objective: To evaluate the adaptation process of ornamental tropical macrophytes irrigated with wastewater, through physiological measurements before and after planting in a tropical constructed wetland (CW).

Design/Methodology/Approach: Three fractions were evaluated with 50%, 75%, and 100% wastewater and natural water (blank) in 0.5×2.0 m fiberglass containers. The following species were placed in the containers during 40 days: *Strelitzia reginae*, *Alpinia purpurata*, *Canna indica*, *Xanthosoma robustum*, *Cyperus papyrus*, *Pistia stratiotes*, *Spathiphyllum wallisii*, *Ruellia brittoniana*, *Pasto pennisetum*, *Solenostemon scutellarioides*, *Iresine herbstii*, *Lantana camara*, *Duranta eracta* golden, and *Asparagus densiflorus*. Subsequently, the individuals —including *Heliconia psittacorum* and *Iris germanica*— were planted and evaluated in a CW after the adaptation period, in order to replace the macrophyte lost during the said period. The following physiological variables were measured: survival percentage, stem thickness, number of flowers, chlorophyll index, and biomass (as a growth variable).

Results: During the first stage (containers), only 11 out of the 14 initial species survived (78.5%), which allowed us to establish which plants had the highest survival capacity in high concentrations of pollutants. These results determined the priority with which these would be planted in the CW.

Study Limitations/Implications: Significant physiological differences were observed ($p \leq 0.005$) in all CW species. *Canna indica*, *Xanthosoma robustum*, *Ruellia brittoniana*, *Alpinia purpurata*, *Cyperus papyrus*, and *Heliconia psittacorum* recorded better adaptation.

Findings/Conclusions: The macrophytes studied show great adaptation as phytoremediative plants in tropical CW systems; however, their physiological development is different.

Keywords: Adaptation, macrophyte, purification, wastewater, constructed wetland.

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INTRODUCTION

About two thirds of Mexico's territory is comprised of arid or semi-arid areas that face natural water scarcity and only one third has a very high relative abundance of water (Arreguin-Cortes *et al.*, 2019). However, runoff from crop irrigation and industrial waste cause water quality degradation, groundwater pollution, sedimentation, and direct toxicity to organisms. Consequently, its impact on biodiversity, fishing, recreation, and public health makes these sources unfit for consumption (Wang *et al.*, 2018; Guan *et al.*, 2019).

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There are many wastewater treatment technologies that seem to be highly efficient; however, they require higher energy consumption and release more carbon emissions, depending on their scale (Chang *et al.*, 2017; Landa-Cansigno *et al.*, 2020). Therefore, constructed wetlands (CW) are a viable and environmentally-friendly alternative for water treatment. CWs consist mainly of shallow ponds in which plant species adapted to aquatic life are implanted and in which purification is based on natural microbiological, biological, physical, and chemical processes. Likewise, their implementation and development are low-cost and they are easy to operate (Li *et al.*, 2018; Liu *et al.*, 2019). Terrestrial ornamental plants are important in these systems, since they provide the main source of oxygen, through a process that occurs in the root zone, known as radial oxygen loss (Sandoval *et al.*, 2019b).

Macrophytes are a wide and varied group of plants that is used for wastewater treatment. In a natural water body, they can be found in different types of growth, along the depth gradient that extends from the edge to the deepest parts (Maine *et al.*, 2009; 2013). In constructed wetlands (CW), an attempt to replicate this type of gradient is made by planting the different types of macrophytes according to how they grow (Figure 1).

The macrophytes in the CW have a thermoregulatory effect that promotes a variety of biological and chemical processes. They increase the filtering effect and porosity throughout the root distribution, they capture and store some essential nutrients in their tissues, and they act as a purification reaction, improving the diversity of the process in the rhizosphere (Türker *et al.*, 2016). One of the main limitations for the development of a plant is the lack of oxygen. Its transportation in an aqueous system is 10,000 times slower than in a porous medium. Therefore, in flooded systems, many plants die due to the lack of oxygen and consequently generate a wide range of responses to stress. The most important adaptation of macrophytes in these conditions is the development of the aerenchyma, a tissue characterized by a large number of air channels that transport oxygen from the aerial part of the plant to the roots (Moreno-Casasola and Waner, 2009; Alarcón-Herrera

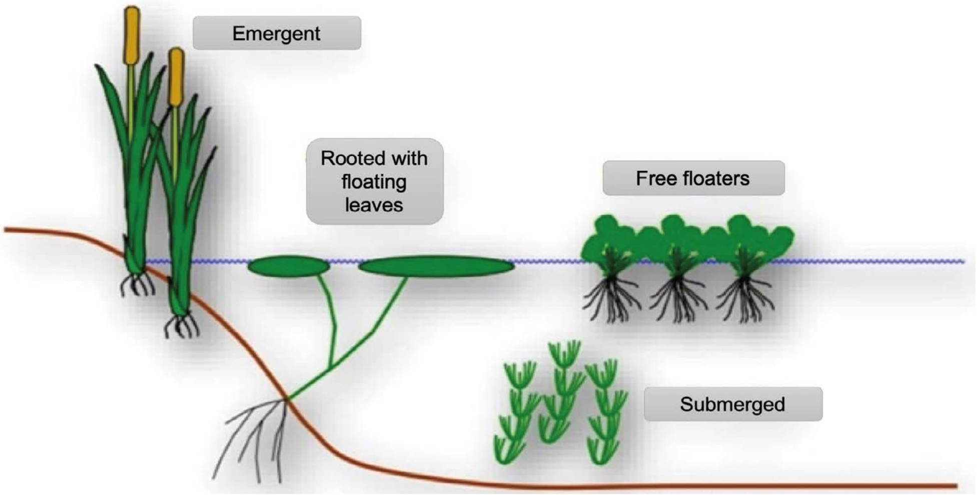

Figure 1. Diagram that represents the different macrophyte life forms (Alarcón-Herrera *et al.*, 2018).

et al., 2018). The uptake rate of plants is also limited by their net productivity (growth rate) and the nutrient concentrations in the tissues. In addition, age greatly influences the physiological activity of plants, especially their roots (Valipour *et al.*, 2015).

Generally, the plants used for CWs in tropical and intertropical areas are macrophytes typical of natural wetlands, such as: *Phragmites australis* and species of the genera *Typha*, *Scirpus*, and *Cyperus* (Sandoval *et al.*, 2019b). However, ornamental vegetation is a promising alternative in CW systems, due to its aesthetic and commercial value, and other aggregates related to biodiversity and ecosystem services. Among the species most frequently used to remove pollutants from CWs, *Canna*, *Iris*, *Spathiphyllum blandum*, *Heliconia*, and *Zantedeschia* stand out (Tejeda *et al.*, 2015; Sandoval *et al.*, 2019; Mateo *et al.*, 2020). These species are highly efficient for the elimination of suspended solids, total phosphorus, ammoniacal nitrogen, and fecal coliforms (Mateo *et al.*, 2020) and contribute to the reusability of wastewater for irrigation purposes. Growth is an indicative parameter of physiological health, carbon assimilation, and adaptation. Consequently, if a plant is stressed by an external agent, long-term symptoms will manifest themselves as lower growth and lower production of leaves, flowers, and stem (Pagter *et al.*, 2005; Heynes-Silerio *et al.*, 2017). The objective of this work was to evaluate the adaptation of ornamental tropical macrophytes to different wastewater concentrations, through physiological measurements. These macrophytes are to be planted in a tropical CW in the central zone of the Gulf of Mexico.

MATERIALS AND METHODS

The study was carried out in the municipality of Boca del Río, Veracruz, Mexico, within the facilities of the Tecnológico Nacional de México/Instituto Tecnológico de Boca del Río (TecNM/ITBoca). The municipality is located in the central coastal zone of the state, at 19° 07' N and 96° 06' W, and an altitude of 10 m (Figure 2).

Figure 2. Geographical location of the municipality of Boca del Río, Veracruz and its surroundings (modified from the Municipal Information System, 2016).

First stage of the experiment

Selection of the ornamental macrophytes

The macrophytes selection was based on the following criteria: 1) easy adaptation, 2) resistance to weathering agents, and 3) commercial interest (Zurita *et al.*, 2006, 2009; Hadad *et al.*, 2007; Maine *et al.*, 2009; 2013; Sandoval-Herazo *et al.*, 2016; Marín-Muñiz, 2017).

The plants were purchased from a local nursery within the municipality of Veracruz. Endemic plants from tropical climates were selected, based on the floristic list of the state of Veracruz (Gutiérrez-Báez *et al.*, 2017). Complete young individuals between 15 and 20 cm high (including flowers) were selected for later adaptation (Table 1).

Macrophyte adaptation

After making holes in their envelopes, the acquired plants were placed in 0.5×2.0 m fiberglass containers filled with water from the natural environment for 10 d to avoid stress. Four water concentrations were prepared: three fractions of wastewater from the ITBoca (50%, 75%, and 100%) and a blank. The wastewater fractions were analyzed before proceeding to the next stage. Plants of each species were placed in each of the concentrations in order to adapt them to the new medium, where they remained for 40 d, maintaining a 15 cm water sheet (Table 1). This adaptation process was carried out from October to December 2019, through the manual insertion of water.

To evaluate the behavior of the selected macrophyte in the flooded systems, the stem thickness was measured with a 0-150 mm Vernier caliper and the chlorophyll index was measured using a SPAD 502 Plus. The survival percentage and the number of flowers were likewise estimated.

Table 1. Selected macrophytes in the adaptation process.

Species		**Number of individuals collected per fraction of wastewater**			
Common name	**Scientific name**	**75%**	**100%**	**50%**	**B**
Platanillo	*Canna indica*	5	5	4	1
Bird of paradise	*Strelitzia reginae*	5	5	4	1
Hawaiian	*Alpinia purpurata*	5	5	4	1
Elegant blade	*Xanthosoma robustum*	5	5	3	1
Papyrus	*Cyperus papyrus*	3	3	2	1
Moses Cradle	*Spathiphyllum wallisii*	26	27	26	1
Coleos	*Solenostemon scutellarioides*	5	5	4	1
Pennisetum grass	*Pennisetum purpureum*	3	3	3	1
Amaranth	*Iresine herbstii*	15	15	14	1
Lantana	*Lantana camara*	10	10	9	1
Duranta	*Duranta erecta golden*	15	15	14	1
Mexican Petunia	*Ruellia brittoniana*	5	5	4	1
Asparagus	*Asparagus densiflorus*	1	1	1	0
Water lettuce	*Pistia stratiotes*	15	15	15	30

Percentage of the concentration of wastewater taken from the ITBoca sump; concentration at 50%, concentration at 75%, concentration at 100%, B; blank with natural water.

Second stage of the experiment

Based on Valles and Alarcón (2014), after the macrophytes had been placed in the acclimatization ponds for 40 d, the second stage began.

Description of the constructed wetland

The second stage of the experiment was developed in a CW with horizontal subsurface flow; water enters the system from a 2,500-L tank that acts as a settling tank. The system operates with a 2-d hydraulic retention time, constantly supplying the system with 31.5 m^3 d^{-1}. It is made up of 7 cells as a treatment train filled with three different substrates: 1) stone material with a 70% porous surface; 2) inert material with 80% porosity (Sandoval *et al.*, 2019a), and 3) calcareous material.

Stocking and evaluation of the macrophyte in the constructed wetland

For this system, the plants used were individuals and suckers that survived the previous adaptation (Table 2). *Heliconia psittacorum* and *Iris germanica* are used to replace the macrophyte lost during the adaptation period. It has a 40-cm growth length. Plants of the same species were cultivated in polycultures, 1 m apart from each other and at a 30-50 cm depth in each cell. The cells were kept outdoors under the normal environmental conditions of the state of Veracruz and afterwards were watered with wastewater from ITBoca.

To determine the influence of the CW in the development of the plant, the temperature was evaluated using an INI-T A12T digital hydrometer and the pH with pH-Fix 4.5-10 reactive strips. Five months later, when the plants were well established in each cell, the number of flowers, stem thickness, indirect chlorophyll, and biomass were measured (Table 2).

The information obtained was subject to an analysis of variance, using the Minitab Version 19 statistical software. A randomized block design and a Tukey test were used to detect significant statistical differences ($p \leq 0.005$) between the physiology of the macrophyte (confidence: 95%).

RESULTS AND DISCUSSION

In the 50%, 75%, and 100% wastewater concentrations, it was possible to observe which of the 14 macrophytes had the greatest ability to survive high pollutant concentrations.

Table 2. Macrophytes that survived the adaptation period, planted in the CW.

Common name	Scientific name	Common name	Scientific name
Strelitzia reginae	Bird of paradise	*Pistia stratiotes*	Water lettuce
Canna indica	Platanillo	*Iris germanica*	Iris
Alpinia purpurata	Hawaiian	*Spathiphyllum wallisi*	Moses Cradle
Heliconia psittacorum	Pennisetum grass	*Pennisetum purpureum*	Pennisetum grass
Xanthosoma robustum	Elegant blade	*Ruellia brittoniana*	Mexican Petunia
Cyperus papyrus	Papyrus		

Consequently, the order in which they would be planted (Figure 3) was determined, based on which plants had the greatest functionality as a phytoremediative plant in CW systems. These results are different from those obtained by Gallegos-Rodríguez *et al.* (2018).

In most cases (including ours), the adaptation of the vegetation is carried out in functioning CWs, in a one-month period (Marín-Acosta *et al.*, 2016). However, according to Valles and Alarcón (2014), this period could vary.

The values of the chemical characteristics of the wastewater used are summarized in Table 3.

Physiological behavior of the macrophyte during the adaptation period

During the adaptation period, only 11 out of the 14 initial species (78.5%) survived. Plant survival percentage for 50%, 75%, and 100% wastewater fraction ranged from 28-100%, 26-100%, and 20-100% respectively. However, during this adaptation period, *Alpinia purpurata*, *Spathiphyllum wallisii*, *Cana indica*, *Xanthosoma robustum*, and *Cyperus papyrus*

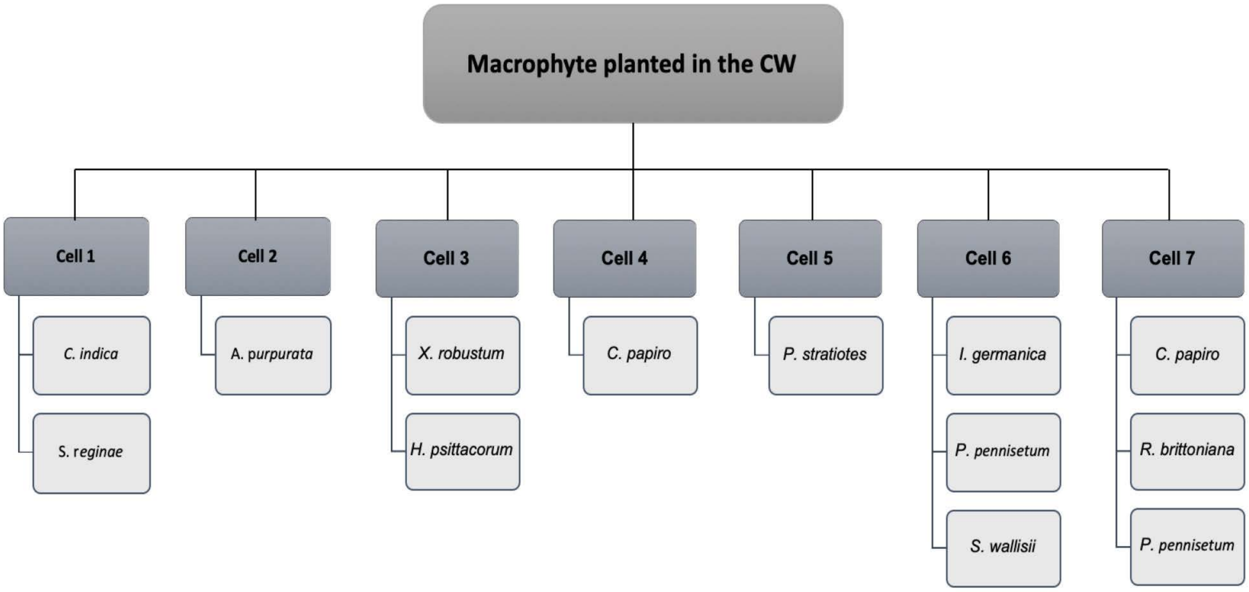

Figure 3. Order in which the macrophytes were seeded in each of the constructed wetland (CW) cells.

Table 3. Characteristics of the TecNM/ITBoca wastewater.

Parameter	Value (mg L^{-1})
Biochemical oxygen demand (BOD)	219.00
Chemical oxygen demand (DQO)	482.15
Total phosphorus (PT)	4.79
Total nitrogen Kjeldahl (NTK)	0.75
Total suspended solids (SST)	75.00
Total dissolved solids (SDT)	845.00
pH	7.90

grew faster and produced a large number of suckers, which indicates that they are more capable of inducing adaptation mechanisms in flooded environments. Most of the plants were healthy, except for the lost vegetation such as *Solenostemon scutellarioides*, *Asparagus densiflorus*, *Lantana camara*, *Iresine herbstti*, and *Duranta erecta* golden, which after a few days showed a less defined state of health.

Among the flowering species, *Spathiphyllum wallisii* produced more flowers (>7 flowers) than *Alpinia purpurata* (≈3-5 flowers) and *Strelitzia reginae* (0 flowers). Although the latter flower is used in artificial wetland systems with domestic waters, it did not develop or flower in any of the wastewater fractions (Arias-Martínez *et al.*, 2010). The coloration of the species showed a direct relationship both with the photosynthetic process carried out during the experiment and with the chlorophyll index, based on which it was compared with the level of pollution of the university's 50, 75, and 100% wastewater fractions. The chlorophyll index was only analyzed in seven species, since species with small leaves could not be read in the infrared, showing a higher chlorophyll concentration at a 75% wastewater fraction in *Ruellia brittoniana*, *Canna indica*, and *Strelitzia reginae* with 51-65, 42-47, and 52-61 SPAD units, respectively. Therefore, planting in the system is recommended after an adaptation period of 40 days.

Physiological behavior of the macrophyte after planting in the constructed wetland

Stem thickness and number of flowers. Regarding the physiological responses of the stem, higher growth rates have been recorded in the first third of the length of the CW in the flow direction than in the rest of the stem, possibly because most of the organic matter and nutrients are consumed in this length (Peña-Salamanca *et al.*, 2013). For this CW, the stem thickness between the same species showed faster growth in the first cells (1, 2, and 3), with significant differences ($p \leq 0.005$) in all species. The *Canna indica* and *Xanthosoma robustum* species are the most representative, with stem thickness of 44.4 and 63.5 mm, respectively, during the 5 months in the CW (Figure 4).

Regarding the number of flowers, *Spathiphyllum wallisii* was the species that changed the most during the adaptation period to the CW system, showing that the conditions in which it was exposed were not favorable. Meanwhile, *Strelitzia reginae* did not produce any flowers, because it was part of a polyculture system, along with *Canna indica*, which showed great development, producing up to 5 flowers per plant (Figure 4).

Chlorophyll index. The fluorescence emission of chlorophyll in plants is a sensitive test of the photosynthetic efficiency of the plant and reflects long-term disturbances in the photosynthetic apparatus. Therefore, the chlorophyll of the vegetation in a wetland can be considered a physiological indicator of plant metabolism and participates in the transformation of the different nutrients in the system (Pérez-Asseff *et al.*, 2007). For this CW system, all species recorded a >20 chlorophyll index ($p \leq 0.05$). The plants with more representative values were: *Ruellia brittoniana*, *Strelitzia reginae*, *Canna indica*, *Alpinia purpurata*, *Heliconia psittacorum*, and *Xanthosoma robustum*. This is a sign of a high acclimatization capacity regarding the pollution conditions to which they were subjected during the study (Figure 5).

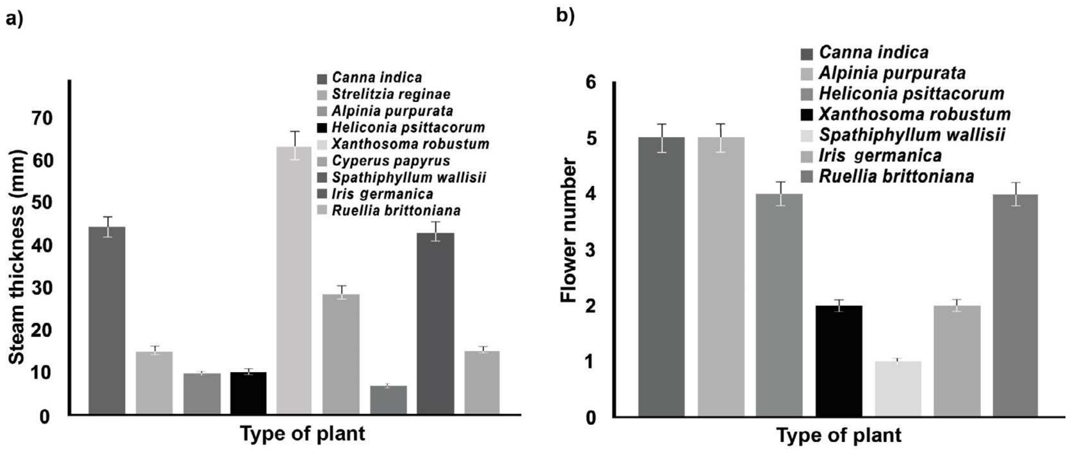

Figure 4. Stem thickness a) and number of flowers b) of the plants in the activated constructed wetland (CW) system.

Biomass. If the root system of the plants has a low development, the soluble or easily assimilated products found in the gradient (height of the substrate) are not absorbed (Ramírez-Cadavid, 2018). Biomass production is shown in three different sections of the plants (root, area, and body). The following species have significantly different values: *C. indica*, *S. reginae*, *H. psittacorum*, *X. robustum*, and *C. papyrus*. These species are suitable for CWs, due to their rapid growth and adaptation. The greatest amount of biomass remains concentrated in the root and the body of the plant, with the exception of *R. brittoniana* and *I. germanica*, where its highest concentration was recorded in the aerial part (6.23 and 12.93 g, respectively). *P. pennisetum* had the lowest biomass production in the three sections (1.51 in the body, 1.2 in the area, and 4.5 g in the root) (Figure 5).

CONCLUSION

Although most of the ornamental macrophytes species survived during the adaptation period, which indicates their capacity to enter flooding mechanisms, each one had different

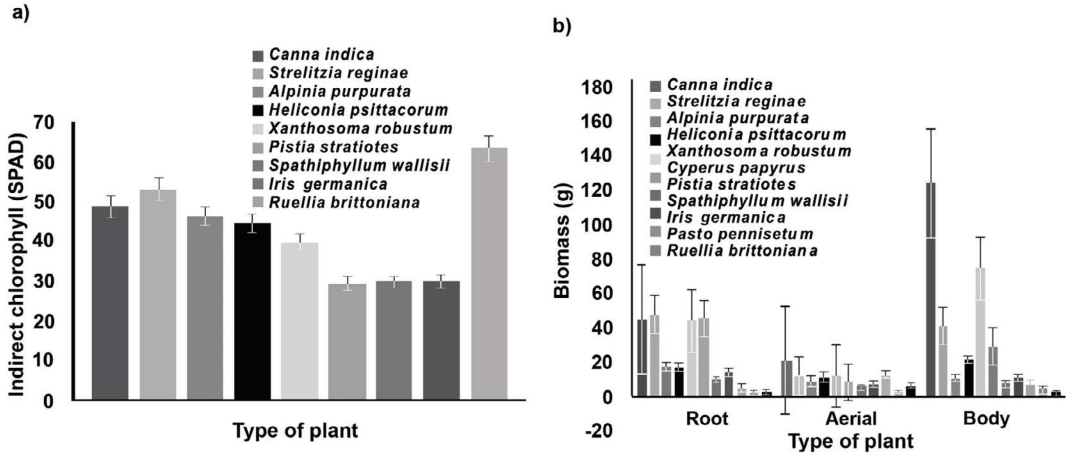

Figure 5. Indirect chlorophyll a) and biomass b) of the plants in the activated constructed wetland (CW) system.

physiological development. Our study obtained information that will serve as the basis for further research about CW systems in the tropical and subtropical areas of the central zone of the Gulf of Mexico.

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