Influence of a new ornamental species (*Spathiphyllum blandum*) on the removal of COD, nitrogen, phosphorus and fecal coliforms: a mesocosm wetland study with PET and tezontle substrates

Luis Sandoval, Florentina Zurita, Oscar Andrés Del Ángel-Coronel, Jacel Adame-García and José Luis Marín-Muñíz

ABSTRACT

The objective of this study was to evaluate the influence of a new species of plant (*Spathiphyllum blandum*) in the elimination of chemical oxygen demand (COD), nitrogen, phosphorus and fecal coliforms (FCs) in mesocosms of wetlands with polyethylene terephthalate (PET) and tezontle substrates under a tropical climate. The experiments were developed at the mesocosm level in 20 experimental units; 10 were planted with *Spathiphyllum blandum*, five in PET substrates and five in tezontle, and 10 more were used as controls without vegetation, of which five contained tezontle and five contained PET. The systems were fed with contaminated water from the river Sordo, with a hydraulic retention time of 3 days for 12 months; samples were taken in the influent and effluents of the mesocosms every 2 weeks, with the purpose of evaluating the removal of contaminants. The results showed that presence of this species tended to improve or significantly improved the removal of COD, NH_4-N, PO_4-P, and FCs by 7%, 16%, 29% and 12%, respectively. It was also possible to confirm that the presence of this species reduced the rate of denitrification. These results confirm that in developing countries it is feasible to find new wetland species to be used for wastewater phytoremediation.

Key words | constructed wetlands, ornamental plants, polluted rivers, tropical climate

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INTRODUCTION

Water is a non-renewable resource that is fundamental for the development of life on the planet; unfortunately, in developing countries such a valuable resource has not received enough attention yet (Bui *et al.* 2018). According to the World Program for the Evaluation of Water Resources of the United Nations (WWAP 2017), in low-income countries (mainly in Latin America) 92% of wastewater generated by different anthropogenic activities is not treated and reaches surface water bodies such as rivers, lagoons and seas without any treatment (Chen *et al.* 2018). Especially in developing countries, 2.4 billion people do not have access to basic sanitation services and consume contaminated water from rivers (Jung 2017).

On the other hand, there are currently many technologies for wastewater treatment such as activated sludge, oxidation ditches, and aerated lagoons that are costly in their implementation and operation (Casierra-Martínez *et al.* 2017). This make them unviable for countries with low economic resources, and even less viable in rural areas where low population densities predominate, availability of large areas of land is common and pollution of surface water bodies is

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frequent due to the final disposal of domestic wastewater (Sandoval-Herazo *et al.* 2018). Therefore, the implementation of ecological and economic technologies that make possible the treatment of wastewater is an urgent need.

In this sense, a viable alternative is the use of constructed wetlands (CWs), which are ecological systems that mimic the function of natural wetlands by improving water quality (Wang *et al.* 2018). Due to their low cost of construction, and easy operation and maintenance, these systems are considered a highly recommended option for wastewater treatment mainly for rural communities in developing countries (García-García *et al.* 2016).

It is well known that a crucial component in CWs is the vegetation, which plays a very important role in the pollutant removal processes and for the proper functioning of the systems. The predominance of high biodiversity in the majority of developing countries allows the use of not only conventional wetland plants but also beautiful ornamental species, some of them with market value. In this regard, some ornamental plants such as *Canna* (Konnerup *et al.* 2009), *Iris* (Yousefi & Mohseni-Bandpei 2010), *Heliconia* (Konnerup *et al.* 2009; Maine *et al.* 2019), *Zantedeschia*, *Anthurium* and *Agapanthus* (Zurita *et al.* 2009) have been already evaluated. In addition, studies with and without plants are still necessary to know their influence on the removal efficiencies of contaminants (Sandoval-Herazo *et al.* 2018; Sandoval *et al.* 2019a, 2019b). Furthermore, most of the research on the use of plants and substrates in CWs has been done using conventional macrophytes of natural wetlands (*Typha, Scirpus* spp.) (Latune *et al.* 2017) and substrates of gravel, tezontle and sand (Zhang *et al.* 2015). There are few studies that consider the use of ornamental plants (Hernández 2016) and even less, the use of polymeric substrates such as PET (polyethylene terephthalate) applied in tropical and subtropical zones, where temperatures are warmer with a greater intensity of sunlight throughout the year, which can favor the rapid development of vegetation and thus accelerate the absorption of contaminants in plants' tissues. With respect to filter materials, new lower-cost substrates are necessary with easily obtained characteristics that do not compromise the processes of decontamination. The use of rough residues of PET bottles avoids disposal of this plastic waste in the environment; the biodegradation period for these bottles is almost 500 years on average (Lou *et al.* 2017). Thus, the use of PET represents a considerable contribution to reduce the impact caused by plastic on the environment, and hence the need to evaluate it.

Taking into account the above, the aim of this study was to evaluate the influence of a new ornamental species of plant (*Spathiphyllum blandum*) in the elimination of chemical oxygen demand (COD), nitrogen, phosphorus and fecal coliforms in mesocosm wetlands with PET and tezontle substrates, from polluted-river waters.

MATERIALS AND METHODS

This study was carried out in the central region of the state of Xalapa, Veracruz, Mexico, (19°30′10″N and 96°52′51″ W) at an average altitude of 1,560 m a.s.l. The climate of the region is humid subtropical with average annual precipitation of 1,436 mm and an average annual temperature of 19 °C.

The mesocosms were built outdoors, and consisted of a total of 20 plastic cylindrical containers (0.36 m height and 0.29 m diameter), of which 10 mesocosms were planted with a *Spathiphyllum blandum* plant (one individual of *S. blandum* was planted in every experimental unit −15–20 cm height. Cuts or prunings were never necessary); five in the presence of tezontle (TZN) (1–1.8 cm in diameter) and five in rough residues of PET (1.5–3 cm in diameter) as substrates. The remaining 10 mesocosms were used as controls with only the corresponding filter medium; five of them contained TZN and five contained PET (Figure 1). The TZN (red volcanic gravel), with a porosity of 58%, was collected from a bank of material near Xalapa, Veracruz, while the PET residues were made from soft-drink bottles collected from a cafeteria in Xalapa and had a porosity of 69%.

The mesocosms (20 L) were established in horizontal subsurface conditions. Hydraulic retention time was adjusted to 3 days in all the experimental units and revised every week (3.5 and 3.1 mL/70 seconds of inflow rate in units with PET and TZN, respectively). Thus, around 115 L of wastewater were treated in units with TZN (five planted and five unplanted units), and 130 L, in units with PET (five planted and five unplanted units) every 3 days. The water was returned to the Sordo River after its treatment.

Once the mesocosms were built, they were fed during the first 30 days with tap water and then a process of adaptation of the vegetation was carried out, applying for 10 days water from the Sordo River mixed at 50%. After, the growth measurements were initiated. The Sordo River is a tributary of the La Antigua River (one of the most important rivers in Mexico) and flows near the facilities where the experimentation took place, with an average water quality index of 40.14%; for this reason it is classified as a polluted urban river (NSF 2010; Olguín *et al.* 2010). This river is the receptor of the direct discharges of untreated wastewater generated

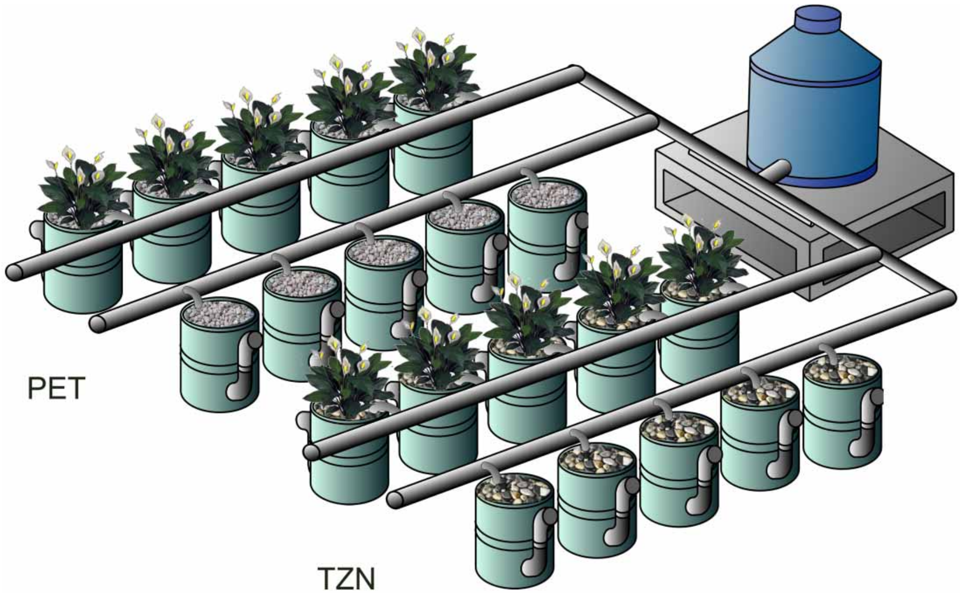

Figure 1 | Mesocosms of wetlands with tezontle (TZN) and polyethylene terephthalate (PET) substrates.

in around 80 settlements (colonies and towns) located along its course. After the period of adaptation, the mesocosms were fed with direct water from the Sordo River, for 1 year (from March 2017 to March 2018).

Physical and chemical parameters

COD, nitrate (NO_3-N), nitrogen in the form of ammonium (NH_4-N), phosphates (PO_4-P) and fecal coliforms (FCs) were determined. The water analysis of the effluent and the mesocom effluents was made every 2 weeks using standard methods (APHA-AWWA-WEF 2005).

The pH and temperature of the water, total dissolved solids (TDS) and electrical conductivity (EC) were measured with a Combo pH and EC waterproof meter. The dissolved oxygen (DO) was measured with a polarographic DO probe with protector (HI76407/F-Hanna), every 15 days during the entire period of study. In a similar way, the ambient temperature and humidity were measured every 2 weeks with a digital hydrometer thermometer (Htc-1 H596) between 12:00 and 14:00. With regard to the plant development, the number of flowers and the height of the plant were measured with a tape measure every 30 days.

Statistical analysis

All data were analyzed using a two-way analysis of variance, using Dunnett's post hoc test to detect significant statistical differences ($P \leq 0.05$) between treatments with respect to pollutant removal. All this was undertaken using the statistical software SPSS version 19 for Windows.

RESULTS AND DISCUSSION

Vegetation development

The environmental conditions during the study favored the development of the plants. The environmental temperature was between 26 and 8 °C and the relative humidity was 48–98%. These values were adequate, according to Paredes & Quiles (2017), who performed a study with *Spathiphyllum lanceifolium* (a plant of the same family as *Spathiphyllum blandum*) and found a satisfactory development under similar conditions of temperature (10–24 °C) and humidity (50–90%) for 12 months.

The maximum height of *Spathiphyllum blandum* (Figure 2) was 104.4 cm in the mesocosm with the TZN substrate, while with PET the maximum height was 107.5 cm. No significant statistical differences were found between the growth of the plant in both substrates ($P > 0.05$). The drop in height was due to the fact that the plant was attacked by a pest that caused damage to some stems, but with natural pesticide the problem was solved and therefore the species recovered completely after December.

The plant managed to adapt and survive adequately in both substrates, although there is no specific reported

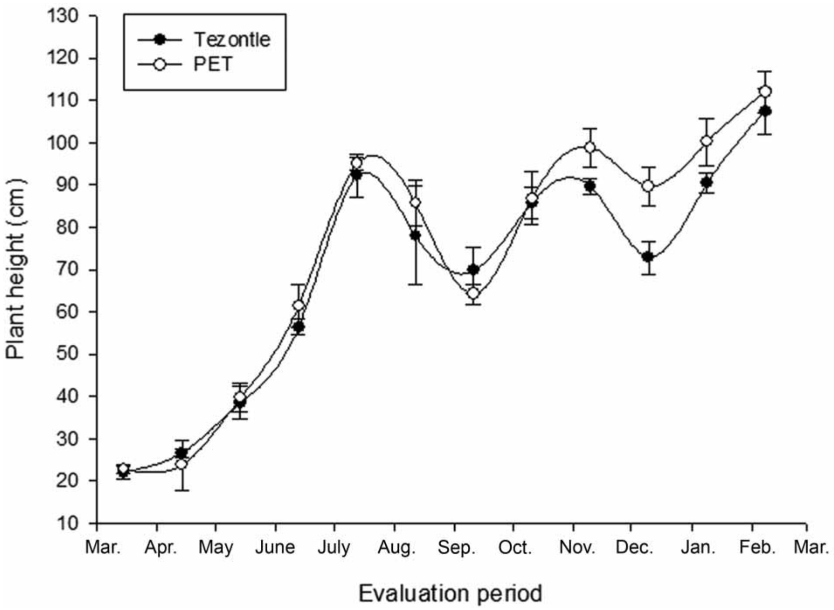

Figure 2 | Growth of the plant in the different substrates.

information regarding the growth of this species. However, in this study the growth of *Spathiphyllum blandum* was similar to that observed in its natural habitat in the Sordo and Actopan river basins. On the other hand, studies using ornamental flowering plants in CWs have reported adaptation processes and growth conditions (Zurita *et al.* 2009; Sandoval *et al.* 2019a, 2019b) similar to those observed with *S. blandum* under CW conditions.

Regarding the production of flowers during the period of study, a significant difference was found ($P < 0.05$) between the two filter media. The mesocosms with TZN produced a higher amount of flowers (a total of 25 flowers) in comparison to those with PET (a total of 15 flowers). On average, the mesocosms with TZN produced 4 ± 2 flowers per plant; while the production of flowers in PET was 3 ± 1 flowers per plant throughout the study. This might be due to the fact that this species of plant needs the presence of some micronutrients such as iron (Fe), magnesium (Mg), copper (Cu), boron (B) and molybdenum (Mo) for greater flowering, since all them are important for the flowering process of the plant (Prieto-Ruiz *et al.* 2013) and they can hardly be found in substrates such as PET, in contrast to TZN where they might be found due to their mineralogical soil characteristics (Yáñez-Ocampo *et al.* 2011).

To the best of our knowledge, this is the first study with the use of this species as emergent vegetation in CWs, so it is necessary to conduct more research to assess its development in conditions of higher concentrations of contaminants. This could be useful to identify other scenarios for its future use in CWs to treat contaminated water.

Wastewater quality

The temperature of the water after passing through the microcosm showed a significant decrease ($P < 0.05$), as shown in Table 1. According to Akratos & Tsihrintzis (2007) the water temperature (between 16.5 and 30 °C) is important for the development of biochemical processes. In this study, the registered water temperatures remained close to the minimum limit for adequate conditions for the development of these processes, since lower values can affect the processes for pollutant removal (Mburu *et al.* 2013).

With respect to TDS in the mesocosms (Table 1), the average value of the influent was reduced in comparison to the value registered in the effluents of the systems, reaching average values of 265.36 ± 72.11 mg/L and 338.84 ± 65.18 m/L, with vegetation and without vegetation, respectively. Significant differences were found not only between the influent and effluents but also between systems with vegetation and without vegetation ($P < 0.05$), as a result of the positive effect of phytoremediation of *Spathiphyllum blandum* as has been found in other studies using different ornamental species such as *Zantedeschia* sp. and *Anthurium* sp. (Tanner 2001; Zurita & White 2014).

As expected and similar to TDS, the EC decreased significantly ($P < 0.05$) in the effluents, both in the

Table 1 | Chemical parameters at input and output of wetland mesocosms

	Wetlands plants in different substrates				
Parameters	**Influent**	***Spathiphyllum blandum* PET**	***Spathiphyllum blandum* TZN**	**PET control**	**TZN control**
Water temperature (°C)	18.7 ± 6.3	16.8 ± 5.2	16.4 ± 4.9	14.8 ± 3.6	15.1 ± 4.4
pH	7.2 ± 0.3	7.3 ± 0.4	7.8 ± 0.3	6.8 ± 0.6	7.1 ± 0.6
EC (μS/cm)	1,826.51 ± 26.18	1,074.48 ± 48.54	1,101.64 ± 78.71	1,254.36 ± 83.55	1,196.72 ± 90.83
TDS (mg/L)	531.11 ± 46.93	267.51 ± 62.41	263.21 ± 81.81	336.51 ± 68.18	341.16 ± 62.17
DO	1.35 ± 0.35	2.91 ± 0.43	2.78 ± 0.54	1.49 ± 0.32	1.57 ± 0.24

Values are given as the average ± standard error ($n = 120$).

mesocosms with vegetation and in the mesocosms without vegetation, registering average values of 1,088.06 μS/cm and 1,225.54 μS/cm, respectively (Table 1). These results could be related to the absorption of ions and macro- and micro-elements through the roots and other tissues of the plants, as well as the physical capacity for TDS adsorption of TZN (Zurita & White 2014) and PET (Sandoval *et al.* 2019b) substrates. On the other hand, the EC values are in the permitted range (<1,500 μS/cm) for cultures sensitive to salt contents.

The values recorded for pH both in the influent and effluents (Table 1) were close to neutral values (6.8 ± 0.6 to 7.8 ± 0.3). These data are consistent with that reported by Justin *et al.* (2009) who indicates that CWs tend to stabilize the pH to neutral values. On the other hand the pH values found in this study are similar to those reported by other authors (Leiva *et al.* 2018; Sandoval-Herazo *et al.* 2018) who have used ornamental plants in CWs systems and have reported values between 6.95 and 7.9. Values in the range of 5–9 are suitable for the development of biological processes and the development of microorganisms that contribute to the purification of pollutants; wastewater whose pH is outside these limits is difficult to treat by biological means (Garzón-Zúñiga *et al.* 2016).

The presence of DO in the systems allows the survival of aerobic microorganisms, which play a leading role in the process of nitrification and in the processes of organic matter biodegradation (Chang *et al.* 2018). The DO values of the effluents in the mesocosms were significantly increased ($P < 0.05$) as can be seen in Table 1, after passing through the systems with vegetation (with respect to the initial data taken from the influent). In contrast, values very similar to that of the influent were measured in the systems without vegetation. Such results in the mesocosms with vegetation could be due to the possible supply of DO by radical action from the plants (Zurita & White 2014).

Removal of contaminants

COD values (Figure 3) collected in the influent were typical of domestic wastewater, on average 211.3 mg/L (Metcalf & Eddy 2003). This is a worrying situation because this clearly indicates that the wastewater from the surrounding settlements is discharged directly to this inter-urban river without treatment. These concentrations decreased significantly ($P < 0.05$) to average values of 52.33 mg/L and 83.41 mg/L after passing through the mesocosms with vegetation and without vegetation, in both TZN and PET, respectively, without difference between the two types of systems, although a clear tendency to lower values was observed in the systems with vegetation. The removal percentages were 78% for TZN and 81% for PET and 69% for TZN and 70% for PET in vegetated systems and non-vegetated systems, respectively. Such results suggest that the presence of *S. blandum* provided an aerated rhizosphere that stimulated the development of a diversity of heterotrophic microorganisms (Wang *et al.* 2019) which were responsible for COD removal. In addition, apparently and surprisingly, this was a little higher in the planted PET units (although without significant difference in comparison to TZN units), probably due to a greater ability of PET for biofilm development (because this trend was also observed in the units without vegetation compared to those TZN units). In general, the results regarding the influence of the vegetation is consistent with the reports of several authors which indicate that no significant differences were found between systems with and without vegetation (Calheiros *et al.* 2007; Zurita *et al.* 2008; Sandoval-Herazo *et al.* 2018). Additionally, this is similar to that reported by Marín-Muñíz (2016) and Sandoval *et al.* (2019b) who used similar substrates. However, apparently, the presence of vegetation in these systems tended to increase the removal of COD by 9% in those systems with TZN and 11% in those with PET.

On the other hand, from the fourth month of operation, the mesocosm wetlands reached a steady state with effluent values around 16.7–12.4 mg/L for wetlands with vegetation and 29.1–29.6 mg/L for wetlands without vegetation (using TZN and PET as substrates, respectively). These low concentrations were in part as a result of lower concentrations in the influent due to the dilution of pollutants as a consequence of higher rainfall during these months. In addition, these results also could be due to the development of microorganisms in the support media and maturity of the vegetation (Knowles *et al.* 2011). On the other hand, it is important to mention that support material has an important role in the adsorption of organic matter; more than 69% of COD was removed in presence or absence of vegetation, and nearly 30% of COD was probably removed by other mechanisms.

Regarding NH_4-N, the concentrations in the influent were observed in the range of 14.5 to 28.4 mg/L, while in the effluents of the systems with vegetation and without vegetation, an average of 18.2 and 22.4 mg/L was found respectively (Figure 3). The main mechanism of removal for ammonium nitrogen is nitrification, which requires the presence of nitrifying microorganisms and oxygen (Lee *et al.* 2009), so in fully saturated systems, as is the case of this study, this process might be limited. In addition, changes in ion concentrations could occur due to the presence of plants.

After going through the treatment systems, the analysis of variance showed significant statistical differences ($P < 0.05$) for the removal efficiencies between vegetated systems (35% for TZN and 31% for PET) and systems without vegetation (18% for TZN and 17.5% for PET). These relatively low efficiencies are expected in this type of wetlands with continuous saturation due to the initial consumption of DO in the biodegradation of organic matter (Li *et al.* 2018). However, it is noteworthy that in systems with vegetation, a better removal of NH_4-N occurred (Figure 3). The presence of *Spathiphyllum blandum* improved the process possibly

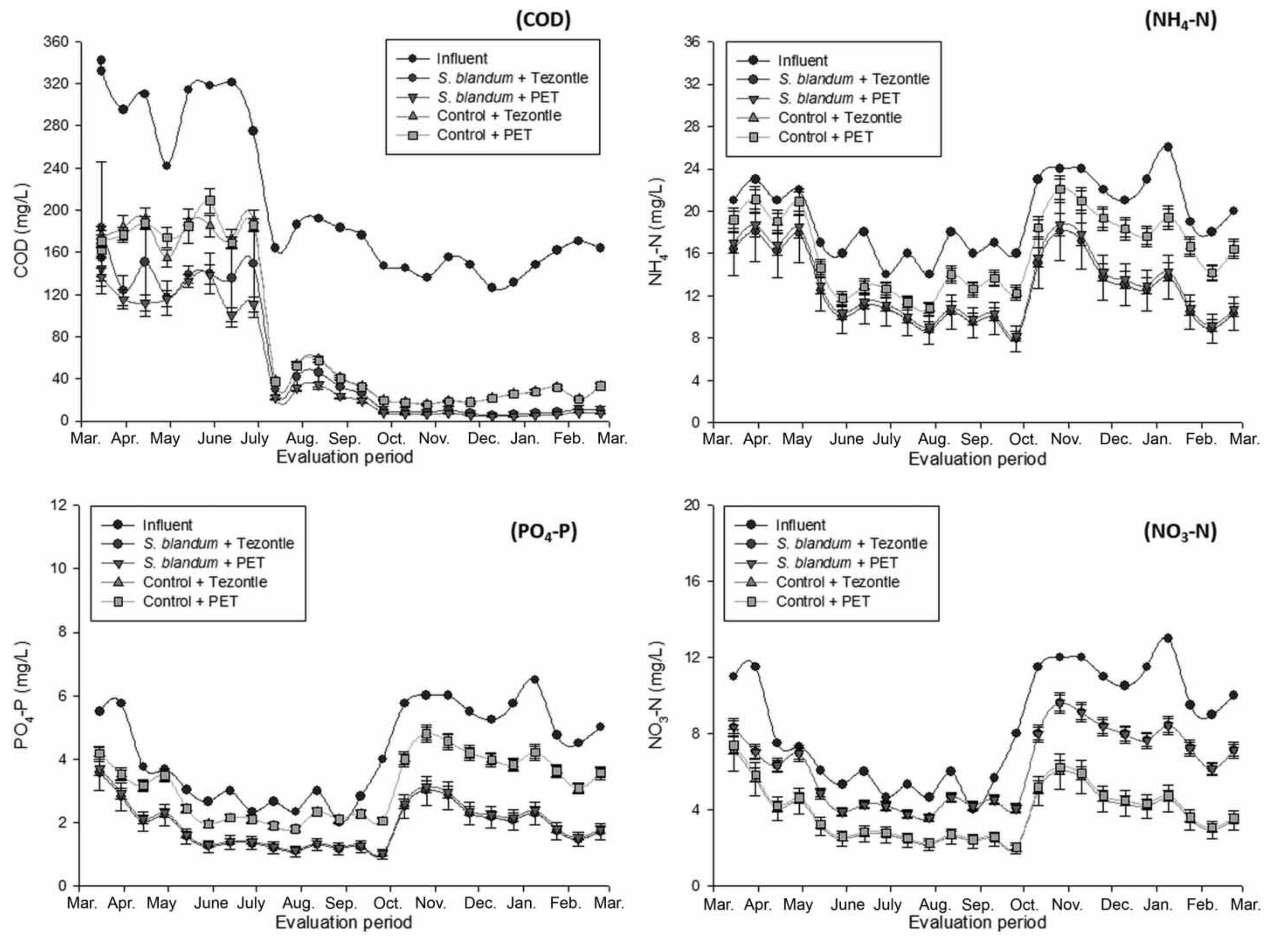

Figure 3 | Concentrations of COD, NH_4-N, PO_4-P and NO_3–N in influent and effluents of mesocosm wetlands using tezontle and PET, with and without the vegetation of *Spathiphyllum blandum*.

due to the oxygen released in the root zone and also due to the direct uptake (Zurita & White 2014).

In contrast to the formation of ammonium, nitrates (NO_3-N) require conditions of absence of oxygen, as well as a source of carbon and denitrifying bacteria (Kleimeier *et al.* 2018). Since the conditions are ideal in the saturated systems of the mesocosms with low presence of DO for nitrate removals (Table 1), in the influent an average of 8.46 mg/L of nitrate was found and it was reduced to average values of 3.85 mg/L and 6.28 mg/L (Figure 4), in the mesocosms without vegetation and with vegetation, respectively. Consequently, significant differences were found ($P < 0.05$) for nitrate removal between vegetated systems (25% TNZ and 26% PET) and non-vegetated systems (55% TZN and 53% PET). This indicates that in the systems where the presence of DO was lower (Table 1), a better reduction of N-NO_3 was possible, which is supported by the fact that these processes are preferably developed in the absence of oxygen (Zhong *et al.* 2019), where nitrate is consumed during the organic matter oxidation; thus the nitrate reduction process could be occurring. In addition, it is well known that in horizontal subsurface flow wetlands, anoxic conditions prevail beyond the root zones, so the denitrification process is possible in vegetated systems.

With respect to phosphorus (PO_4-P), the main mechanisms of elimination in CWs are adsorption onto substrates, precipitation, uptake by plants and microbial action (Casierra-Martínez *et al.* 2017); however, the use of PET as a substrate can limit the adsorption mechanism, probably due to the smooth wall of PET waste compared with the TZN texture; this was corroborated comparing statistical analysis about pollutant removals between substrates and vegetation presence. Figure 3 shows the dynamics of the behavior of PO_4-P; the concentrations were low, with an average value in the influent of 4.23 mg/L that was reduced to average values of 1.93 mg/L and 3.14 mg/L in the mesocosms with vegetation and without vegetation, respectively. In this way, significant differences ($P < 0.05$) were found between systems with vegetation (53% TZN and 51% PET) and without vegetation (24% TZN and 20% PET) for the removal of phosphates. This reduction could be due to the uptake of nutrients by the plants for their development (Casierra-Martínez *et al.* 2017); generally, this nutrient requirement tends to be greater in tropical zones (García *et al.* 2008) like the region under which this of study was carried out. In addition, the nutrient uptake by the vegetation tends to be higher with a fast rate of growth (Vymazal 2007) as happened with this species (Figure 2). On the other hand, there were not significant differences in the removal of pollutants between TZN substrate and PET, indicating the importance of phytoremediation mechanisms on phosphate removal.

Finally, FCs are one of the main pollution indicators used to determine the presence of pathogenic organisms in contaminated waters (Garzón-Zúñiga *et al.* 2016; Adrados *et al.* 2018). Figure 4 shows the behavior of FCs in the influent and effluents.

The elimination of FCs in CWs is due to several mechanisms, such as sedimentation when they are associated to

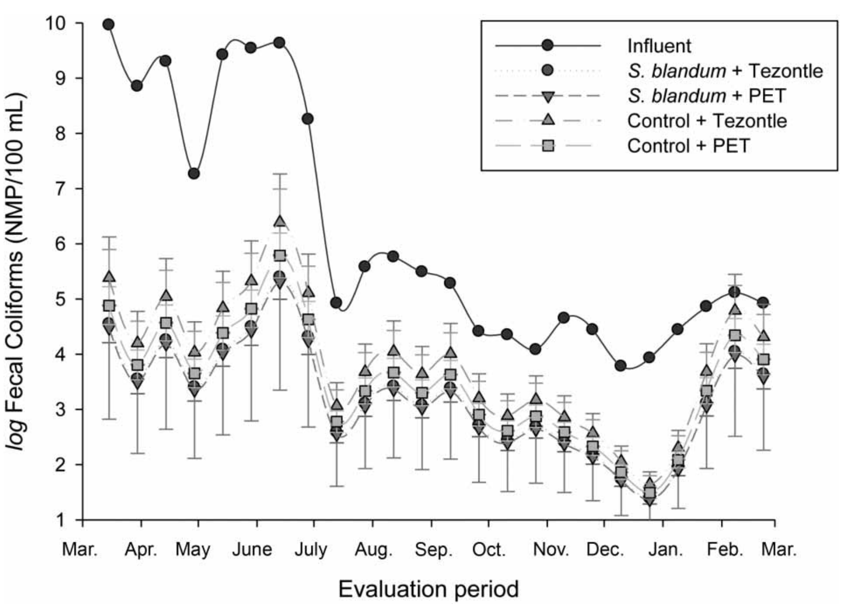

Figure 4 | Fecal coliforms (NMP/100 mL) in influent and effluents of mesocosm wetlands using tezontle and PET, with and without the vegetation of *Spathiphyllum blandum*.

suspended solids, oxidation, adsorption on the filter medium and root biofilms, natural decay and the bactericidal effect of the exudates released through the radical zone of plants (Zurita Martínez & Torres Bojorges 2018). In this study, although there were no significant statistical differences between the mesocosms with and without vegetation ($P > 0.05$) (as shown in Figure 4), a tendency to a positive effect of the vegetation, *Spathiphyllum blandum* (62% for TZN and 56% for PET), was observed. This suggests that this species contributed to the removal efficiency through the creation of an aerated rhizosphere and associated biofilm development and the release of exudates that negatively affected the growth of FCs. It has been demonstrated in other studies with CWs, that the higher the density of vegetation, the greater the removal of FCs (Zurita & Carreón-Alvarez 2015). The removal values in the vegetated systems were 9% (TZN) and 4% (PET) higher than for the systems without vegetation. On the other hand, although FCs were reduced in the vegetated systems, the removal percentages were not enough to produce a reclaimed water to be used in agriculture. The low water temperatures along the period of experimentation could be one of the reasons for the low reduction of FCs, since at lower water temperature, the reactions that contribute to the mortality of pathogenic microorganisms tend to occur at low speed (Al-Maliky *et al.* 2018). In addition, Zurita & Carreón-Álvarez (2015), in a pilot-scale study demonstrated that at least two stages are required to produce reclaimed water to be used for irrigation. Research is needed to evaluate this species in systems with longer hydraulic retention times as well as in climatological conditions of higher temperature that favor the elimination of FCs.

CONCLUSIONS

The evaluation of *Spathiphyllum blandum* species as an emergent vegetation to be used in CWs showed that this species easily adapted to the CWs conditions, improving or tending to increase the removal of COD, NH_4^+-N, PO_4-P and FCs; probably with a longer period of experimentation it would be possible to obtain more conclusive results. Thus, this study revealed the phytoremediation process of *S. blandum*. It was also possible to confirm that the presence of this species reduced the rate of denitrification. In this way, it would be recommendable to use this species in large-scale systems in this subtropical region of Mexico. Additionally, other studies with wastewater with higher concentrations of pollutants are necessary to better understand the capacity of this species as a feasible plant for CWs, as well as studies to evaluate the biofilm acting on the support medium and/or rhizosphere zone, its uptake capacity and accumulation of pollutants in aerial parts and roots. *Spathiphyllum blandum*, in addition to being a plant in tropical areas, gives an added value to the systems, both for its aesthetic beauty and for the exoticness of its flowers. Therefore, future studies could focus on assessing the potential of the species for market purposes and as an added value in CWs. Finally, the favorable effect of rough residues of PET in CWs makes them a viable alternative to be used as a substrate in wetland systems of subsurface flow, giving place to a second sustainable use for these wastes, which have become an environmental problem due to their long periods of biodegradation of over 500 years. According to this study, the use of *S. blandum* and PET could be convenient in future CW designs.

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