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# Effect of hydraulic loading rate and vegetation on phytoremediation with artificial wetlands associated to natural swimming pools

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## Abstract

The treatment of pool water, whether for recreational or sporting purposes, by phytoremediation is widely applied. This work evaluates two artificial vertical flow wetlands, one on a real scale and the other on a laboratory scale, which have been planted with *Typha domingensis*, for the treatment of pool water in the climatic conditions of the city of Santiago de Cuba. When the hydraulic load applied to the real scale wetland was less than  $0.25 \text{ m}^3 \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ , the levels of organic and microbiological contamination in the pool were below the maximum limits allowed by Cuban standards. At a laboratory scale, the presence of vegetation favoured the elimination of nitrogen compounds (nitrates and ammonium) and organic materials (BOD and COD). This behaviour is explained by the presence of processes of assimilation of organic compounds, or by the action of microorganisms associated with the rhizome of plants, which establish a symbiotic mechanism favourable to phytodepuration. The minimum concentration of ammonium obtained in outflow from the laboratory-scale reactor without vegetation reached a value of  $2.15 \text{ mg} \cdot \text{m}^{-3}$ , which is within the limits allowed by the sanitary regulations.

**Key words:** artificial wetlands, natural swimming pools, phytoremediation, sub-surface flow, *Typha domingensis*

## INTRODUCTION

Swimming is a highly recommended sport because it has potentially beneficial effects on human health and, in general, on personal well-being. A large sector of the

population uses swimming pools for various activities within the sports, recreational or medical environment, even when in these environments people are prone to contracting various types of diseases [POND 2005]. Different types of pathogenic and opportunistic microorganisms that

are present in the swimming pools can penetrate the body of bathers, either by direct ingestion of water or by other indirect means, causing skin diseases, gastrointestinal illnesses and/or respiratory disorders [WHO 2006]. In addition with the pollution inherent to the sources from which the swimming pool waters come, the main sources of contamination in the swimming pools come from the bathers themselves: i.e. body excretions, lotions and cosmetics, among others [TEO *et al.* 2015].

Several investigations have been carried out on the chemical contamination of swimming pools, which have focused mainly on means to save the cost of disinfectant products [RICHARDSON *et al.* 2010; YANG *et al.* 2016; ZWIENER *et al.* 2007]. The fundamental priority of the disinfection process in the swimming pools is to maintain the microbiological quality of the water to inhibit the spread of infections and diseases. In order to meet this objective, high concentrations of sodium hypochlorite are commonly used in swimming pools to ensure a water free of microorganisms [RICHARDSON 2010]. Likewise, with the increasing concentrations of chemical compounds in swimming pool waters, much attention has actually been paid to their effect on bathers. Most of the countries, however, have not yet specified the maximum concentration limits of chemical compounds for the disinfection of swimming pool waters, but some countries, such as Germany and France report maximum levels of trihalomethanes (THMs) of 20 and 100  $\mu\text{g}\cdot\text{dm}^{-3}$ , respectively [FLORENTIN *et al.* 2011]. Therefore, it is necessary to reduce the levels of chemical contaminants through alternative and emerging methods that improve the quality of swimming pool waters [CHOWDHURY *et al.* 2014].

Natural swimming pools (NSP) are sources of water which are separated from surface-water and ground-water by artificial constructions. The fundamental characteristic of these pools is that they do not require any chemical disinfection, taking the advantage of their capacity of recovery and constant flow of water. The treatment of the water is carried out through mechanical techniques, biological filters and plants growing in the system. Currently, the demand for natural swimming pools has increased significantly in the world. This increase is due to several factors, i.e.: i) the elimination of chlorinated compounds and other chemical compounds for the disinfection of water; ii) the reduction of toxic compounds formed by the reaction of disinfectants and natural organic matter, process called disinfection by products (DBPs); iii) the aesthetic appeal of natural pools due to the attractive designs used; and iv) the low costs of construction, operation and maintenance compared to other available methods [CASANOVAS-MASSANA, BLANCH 2013]. Also, the use of this technology is adequate to overcome the disadvantages of conventional swimming pools, such as: high lifetime costs, more chemical use, more maintenance and high initial costs [ARROYO *et al.* 2015].

The technique of phytodepuration is widely used to improve the chemical-microbiological quality of NSPs. It is a simple treatment technology that only requires a pumping equipment for the water recirculation; however, the pumping system can be suppressed depending on the natu-

ral pool mode. In general, for the construction of a phytodepuration system local materials are used, which constitute the support material of the filtration zone. The type of phytodepuration technique most commonly used in natural pools is the artificial wetland (AW), which consists of an area with the soil saturated with water in which a community of microorganisms adapted to aquatic life lives in association with different plant species [BERNINGER *et al.* 2012; GUITTONNY-PHILIPPE *et al.* 2014; SULTANA *et al.* 2014; VYMAZAL 2014; WU *et al.* 2015].

Nowadays, the construction of artificial wetlands is based on two systems: artificial wetlands with free water surface (AW-FWS) and artificial wetlands with sub-surface flow (AW-SSF). Free water surfaces are similar to natural wetlands, with a shallow wastewater stream circulating on a saturated substrate; while in the sub-surface flow, the wastewater flows horizontally (AW-HSSF) or vertically (AW-VSSF) in a filtering zone which allows the growth of the plants, allocated in the flow direction [JÓZWIAKOWSKI 2017; SAEED, SUN 2012]. Currently, several studies have focused on the design, development and operation of AWs, which have reported that the latter are efficient in the removal of several pollutants (organic matter, nutrients, trace elements, pharmaceutical contaminants and pathogens) on the wastewaters [BADHE *et al.* 2014; CUI *et al.* 2010; HARRINGTON, SCHOLZ 2010; MIODUSZEWSKI 2014; SAEED, SUN 2013; SKRZYPIEC, GAJEWSKA 2017; YALCUK, UGURLU 2009]. However, the suitable and sustainable operation of the AWs remains a challenge since the operational parameters are essential in the removal efficiency of the system [WU *et al.* 2014], but the plant species and the different filters' substrates may also have a significant influence on the elimination of pollutants [ARIAS *et al.* 2001; LI *et al.* 2008].

Current research indicates that progress has been made in the design and operation of AWs which has increased pollutant removal efficiency, as well as the sustainable application of this treatment system. For example, excellent performance of AWs has been achieved on the treatment of highly pollutant wastewaters, even in temperate environmental conditions, with an adequate mode of operation and handling of the hydraulic and organic loading rates; and with the possibility of selecting both the plants and the filter medium. Due to the increase in environmental legislations on water quality, there are still some limitations in the application of AWs for the treatment of wastewaters. Therefore, research is still required to increase the knowledge in the critical aspects of this technology. Some of these critical considerations are summarized below: i) since plants and filter media are critical factors for the adequate removal of pollutants from wastewater in AWs, the appropriate selection of macrophyte species to be used is very important because they affect the production of plant biomass, the emission rate of oxygen and carbon compounds, the removal rates of heavy metals and inorganic compounds, the tolerance to handle high loads of pollutants and the effect on the efficiency of the system not only in one season but in different seasons; ii) because the optimal operation of the AWs depends on the environmental conditions and on the hydraulic loads applied, knowing

the appropriate settings of these two factors still demand even more in-depth studies; iii) nutrients and other contaminants assimilated by plants can be released into the water when the plants die; therefore, appropriate management strategies are required for nutrient recycling in the AWs [WU *et al.* 2015].

The most frequently used macrophytes species in AWs include emerged, submerged and floating plants on the water surface. Actually, there are reports of more than 150 of macrophytes species that have been used in wetlands [VYMAZAL 2013]. Among the emergent species commonly used are: *Phragmites* spp, *Typha* spp, *Scirpus* spp, *Iris* spp, *Juncus* spp and *Eleocharis* spp. The submerged plants most frequently used in the AWs are: waterthyme (*Hydrilla verticillata*), coontail (*Ceratophyllum demersum*), tape grass (*Vallisneria spiralis*), whorl-leaf watermilfoil (*Myriophyllum verticillatum*) and curly-leaf pondweed (*Potamogeton crispus*); and some the floating plants are of the species water lilies (*Nymphaea tetragona*), water fringe (*Nymphoides peltata*), water chestnut (*Trapa bispinosa*), European watercress (*Marsilea quadrifolia*), common water hyacinth (*Eichhornia crassipes*), floating fern (*Salvinia natans*), frogbit (*Hydrocharis dubia*) and common duckweed (*Lemnaminor*) [WU *et al.* 2015].

The main vegetation used in AW-FWs and AW-SSF bioreactors for wastewater treatment is emergent macrophytes. In particular, *Phragmites australis* is the most widely used in Europe and Asia; *Typha latifolia* in North America; *Cyperus papyrus* and *Scirpus validus* in Oceania; whereas, *P. australis*, and *Typha domingensis* are the predominant species in South America [VYMAZAL 2011]. Actually, *Thypha sp.*, has deserved a lot of attention for its usefulness in several ecological aspects, such as improving water quality in treatment systems [COVENEY *et al.* 2002; GOMES *et al.* 2013; HEGAZY *et al.* 2011; SCHRODER *et al.* 2003], restoring the diversity in wetlands [BOERS *et al.* 2007; NEWMAN *et al.* 1996], influencing greenhouse gas emissions in wetlands [CHANTON *et al.* 1993; GROSS *et al.* 1993] and nutrients recycling [SHARMA *et al.* 2006].

Several European countries, for example, Spain, Portugal, Germany and Switzerland, have many years of experience in the application of phytodepuration methods with AWs for swimming pool water treatment. However, there is still no European regulatory directive for the design, construction and management of NSP; although several of them have been approved in different countries by the corresponding national health authority [MARION *et al.* 2010]. In Latin America, countries like Mexico, Brazil, Argentina and Colombia have entered this area, and currently several of these technologies are under development and homologation [GERALDES *et al.* 2014]. According to studies carried out by CASANOVAS-MASSANA and BLANCH [2013], it was emphasized that a natural purification system may be insufficient to reduce the pathogen load. For this reason, it is necessary to continue investigating this type of swimming pool in order to determine the parameters that guarantee an acceptable microbiological risk for users. Therefore, the objective of the following work was to evaluate the use of artificial wetlands with vertical sub surface flow (AW-VSSF) to treat the water of a natural swimming pool

and to study the effect of the operating factors (hydraulic load and vegetation) that influence the elimination efficiency of pollutants.

## MATERIAL AND METHODS

### CHARACTERIZATION OF THE FILTER MEDIUM

Materials used in the filter media for the AW-VSSF bioreactors were washed sands and gravels from two quarries located in the province of Santiago de Cuba (20°01'11.4" N; 75°48'50.1" W), Cuba. Two volumes of 0.25 m<sup>3</sup> of sand and gravel, respectively, were removed and deposited in polyethylene bags for transport to the laboratory. Afterwards, a small part of both materials (10 kg) were used for their characterization following the methodology specified in the Cuban Official Standards (NC): effective diameter, uniformity coefficient, shape factor, maximum and minimum diameters of the materials, organic impurities, specific weight and water absorption, percentage of voids, and content of clay particles. Hardness is the opposition that materials offer to external alterations, such as penetration, abrasion, scratching, shearing, and permanent deformations. The hardness of the filter material (gravels) was determined by the Mohs scale method, which consists of a hardness test based on the resistance offered by a non-metallic material to be scratched. Acid erosion was carried out by direct contact with different types of acids: hydrochloric acid (HCl), sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and nitric acid (HNO<sub>3</sub>) for 24 hours. Then, the percentage of wear of the filter material used was determined.

### COLLECTION AND PREPARATION OF THE WATER TO BE TREATED

The waters used in the biodegradation experiments were collected from different surface water sources, commonly used for recreational purposes in the territory of Santiago de Cuba. After the water was collected, it was transported in 20 dm<sup>3</sup> plastic containers to the site where the experiments were conducted.

### LABORATORY-SCALE BIOREACTORS

An outline of the experimental layout used to assess the use of AWs for the treatment natural swimming pools can be seen in Figure 1. Two AW-VSSF bioreactors, called R1 and R2, were used in the experiments. Both bioreactors were constructed with red ceramic tubes with an internal diameter of 0.1 m and 1.0 m in height, so that the area of their cross-section and their total volume was 0.0078 m<sup>2</sup> and 0.078 m<sup>3</sup>, respectively. The sand and gravels used in the experiments were washed with water, prior to their placement in the bioreactors. Then, disinfection with sodium hypochlorite (NaHCO) diluted at 2 mg·dm<sup>-3</sup> was performed for 30 minutes to avoid any possible bacterial contamination. Next, the bioreactors were washed with sterile distilled water. Inside each bioreactor was placed a granular filter bed composed in ascending order of three layers of different granulometries: the first one of 100 mm in

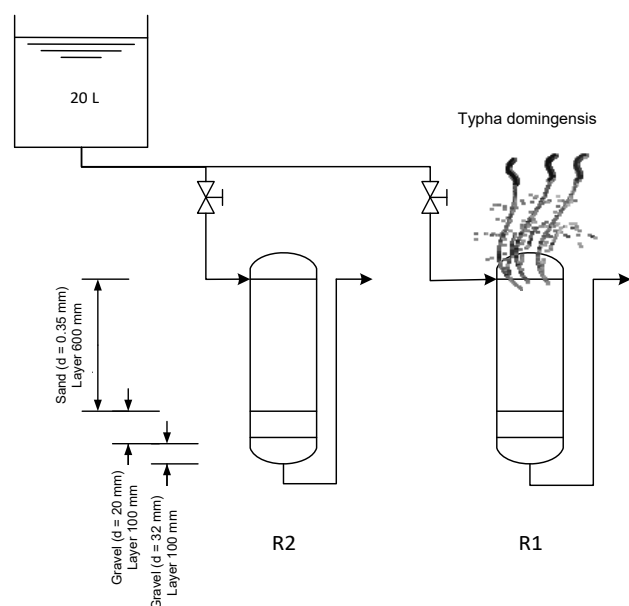


Fig. 1. Layout of the AW-VSSF bioreactors built on a laboratory scale. The vegetation used in the bioreactor on the right (R1) was *Typha domingensis*; bioreactor on the left (R2) had no vegetation;  $d$  = substitutive grain diameter; source: own elaboration

height consisted of gravel having an effective diameter of 32 mm; the second layer of 100 mm in height consisted of gravel having an effective diameter of 20 mm; and the third layer of 600 mm in height consisted of washed sand with an average effective diameter of 4.76 mm, 32% of voids and whose coefficient of uniformity was 4. The depth of the water column in both bioreactors was 0.8 m.

In the bioreactor R1, rhizomes of *Typha domingensis* were planted as emergent aquatic plant; whereas in the bioreactor R2 no vegetation was planted. In each bioreactor, a high-density polyethylene (HDPE) pipe, with an internal diameter of 6.0 mm and a length of 1.0 m, was installed at the bottom as an outlet conduit and for sampling. In addition, a 20 dm<sup>3</sup> capacity reservoir, having two serological hoses with their corresponding valves, supplied a constant flow and hydraulic load to the bioreactors. During the acclimatization period (30 days), both bioreactors were washed with a continuous and uniform flow rate of drinkable water for one hour a day.

#### ACCLIMATIZATION AND GROWTH CONTROL OF *Typha domingensis*

Several specimens of *Typha domingensis* were collected in a natural wetland near the city of Santiago de Cuba. Planting of the plant material in R1 bioreactor was performed by extracting a rhizome from the harvested plant. The rhizome was extracted with the use of a pickaxe; while the leaves were cut with scissors. The remaining vegetable material was washed with water to remove the substrate residues and disinfected with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Once the rhizome was seeded, R1 bioreactor was maintained in a continuous and uniform irrigation regime with drinkable water free of residual chlorine for 24 h. After this time, irrigation was carried out for 1 h per day. The acclimatization time was adjusted to 1 month.

Once the first leaves of the *Typha domingensis* were visible, the plant and leaf growth rate was recorded measuring the length and width of the stem and the number of leaves and their dimensions. This step allowed evaluating the adaptability of the plant to the support medium and its vegetative development in the experimental conditions. Plant growth control was performed in two stages: one at the laboratory scale and other at the pilot scale. In the first stage, vegetation growth rate, quantity and width of leaves, vegetation cover and wet weight of each plant were determined; in the second stage, the same variables were measured but in a larger surface area (1 m<sup>2</sup>), with the objective of assessing the development of vegetation in a pilot-scale.

#### EXPERIMENTAL DESIGN

The experimental design was a factorial design in which the variables to be assessed were surface hydraulic load, vegetation, water sources and samples; while, the other parameters of the model characterized the effect of the interactions between the aforementioned factors, respectively. The levels of the factors or variables assessed were defined as follows: surface hydraulic load (0.25 and 0.50 m<sup>3</sup>·m<sup>-2</sup>·d<sup>-1</sup>), vegetation (with vegetation and without vegetation), and 3 levels of water sources. The statistical analysis was carried out with the ANOVA procedure of the statistical package Statgraphics Centurion XV (Statpoint Technologies, 2007). Differences between main effects means, and their interactions, were assessed by a least significant difference (*LSD*) test ( $P < 0.05$ ).

#### EXPERIMENTAL PROCEDURE

After a period of acclimatization of 30 days, considering this time necessary for the growth of the vegetation in R1 bioreactor and for the formation of biofilms of microorganisms in R2 bioreactor, the evaluation of the different conditions of the experiment was initiated. The bioreactors were filled in two consecutive days with the water collected from the sources, applying continuously a water flow between 0.002–0.004 m<sup>3</sup>·h<sup>-1</sup> during 8 h·day<sup>-1</sup>. These values range agree with the designing criteria for multiple and slow filters of sands used for water disinfection [USEPA 2000]. The samples for the physical-chemical and microbiological indicators of the waters treated were carried out in duplicated; samples of the effluent treated was collected every week during 1 month.

#### PHYSICAL-CHEMICAL AND MICROBIOLOGICAL CHARACTERIZATION OF THE WATERS

The physical-chemical and microbiological characterization of the waters used in the experiments was completed according to the Standard Methods for the Examination of Water and Wastewaters [APHA 1995]. The analyses of the water samples used were performed in the Laboratory of the National Institute of Hydraulic Resources (INRH) and in the Center of Studies of Industrial Biotechnology (University of Oriente, Cuba). Concentrations of chemical oxygen demand (COD) and biological oxygen demand (BOD), nitrates, ammonia and pH were determined. The determi-

nation of total and faecal coliforms was done in the Laboratorio de Higiene y Epidemiología del Ministerio de Salud Pública (MINSAP), Santiago de Cuba, following the procedure of counting by dilution (number more probable). To differentiate the bacterial populations of total coliforms from those of faecal coliforms, the water samples were incubated during fermentation of the lactose at 37°C and 44°C, respectively [MCCRADY 1915].

## RESULTS AND DISCUSSION

### PHYSICAL-CHEMICAL AND MECHANICAL CHARACTERIZATION OF THE FILTER MEDIA

The different components characterizing the sand used in the filter bed of the bioreactors were: 43% quartz; 27% feldspar; 5% calcite; 13% montmorillonite minerals; and 5% of rock remains. According to the standard used, this type of sand is defined as natural, i.e. sand that comes from the natural disintegration of the rocks. Resistance to the compression showed minimum and maximum values of 800 and 1200 kg·cm<sup>-2</sup>, respectively. Dry saturated weight was 2.55 g·cm<sup>-3</sup>, wet saturated weight 2.61 g·cm<sup>-3</sup> and the absorption 2.8%. The swelling coefficient ranged from 1.5 to 1.8, with a hardness of 6 according to Mohs scale. Table 1 shows the wear percentage of the gravels used in the filter bed after being exposed to the action of different acids for 24 hours. Gravel with a mean effective diameter of 32 mm had the highest percentage of wear, 15.5%, when diluted with hydrochloric acid (HCl), while the lowest percentage of wear, 1.71%, was that of gravel with a mean effective diameter of 20 mm when diluted with nitric acid (HNO<sub>3</sub>). Both values were considered acceptable for being

**Table 1.** Wear percentage of filter media (gravels)

Grain diameter	Acids (diluted to 5%)	Initial weight (g)	Final weight (g)	Wear (%)
20 mm	hydrochloric acid (HCl)	11.792	10.794	8.97
	sulfuric acid (H <sub>2</sub> SO <sub>4</sub> )	28.836	28.155	2.36
	nitric acid (HNO <sub>3</sub> )	22.850	22.460	1.71
32 mm	HCl	30.000	25.500	15.50
	H <sub>2</sub> SO <sub>4</sub>	30.000	28.365	5.45
	HNO <sub>3</sub>	30.000	29.250	2.50

Source: own study.

used as filter media. This material is quite resistant to the action of acids and to all sort of mechanical actions, because they are granitoid materials and they are considered as hard, hardness of 6 in the scale of Mohs. This feature makes it a suitable material to be used as filter media in wetlands; for this reason, it has been recommended by the United States Environmental Protection Agency [USEPA 2000].

The diameter of the particles is one of the main parameters that influence the effectiveness of the filtration or the retention of suspended solids in the AWs. Fine aggregates, such as sand, with an effective diameter between 0.25–2.0 mm, are the most used media filters in the elimination of pathogens and nutrients. However, they tend to reduce the porous space, making it difficult for water to pass through and are prone to clogging the filter [BRIX, ARIAS 2005]. On the other hand, coarse gravel, with effective diameters greater than 2.0 mm, allows pores to be larger, water to drain without any difficulty and the filter not to be easily clogged [KNOWLES *et al.* 2011]; but the surface area in the upper layers of the filter medium is inadequate for the growth of the microbial biofilm.

There are other materials, such as zeolite and activated carbon, for example, which have a diameter similar to sand but with a porous structure that ensures a large surface area for the development of biofilms. The greater this surface area, the greater the capacity of adsorption and desorption of pollutants from wastewater [DORDIO, CARVALHO 2013]. Another important factor to consider is the hardness of the material that constitutes the filter medium. Hard materials, with values higher than 6 on the Mohs scale, are able to withstand the physical actions of sieving and the impacts due to inertial effects during the filtration process. Likewise, clay has been used in AW-SSF with promising results in the removal of metals and pathogenic microorganisms present in industrial wastewater [GONZÁLEZ 2006].

### CHARACTERIZATION OF THE WATERS USED

The characterization of the water used in the laboratory experiments is shown in Table 2. A wide variability was observed in the chemical parameters of the sampled waters, depending on the influx of people to these water sources and the actual flow of the sources. As these factors

**Table 2.** Characterization of waters used for laboratory tests using WA-VSSF bioreactors

Parameter	Unit	Natural waterfall Juan González		Carpintero river		Porvenir swimming pool		Average value	Standard deviation
		M1	M2	M1	M2	M1	M2		
<b>Chemical characteristics</b>									
BOD	mg·dm <sup>-3</sup>	2	5	7	6	6	2.5	<b>4.75</b>	±2.0
COD	mg·dm <sup>-3</sup>	8	18	22	21	22	9.6	<b>16.7</b>	±6.4
Nitrate	mg·dm <sup>-3</sup>	0.5	0.85	<2.08	<2.08	<2.08	2.5	<b>1.7</b>	±0.8
Ammonia	mg·dm <sup>-3</sup>	0.6	1.25	7.63	7.87	7.9	2.5	<b>4.6</b>	±3.5
pH	–	7.5	7.5	7.5	7.5	8.0	7.0	<b>7.5</b>	±0.1
<b>Bacteriological characteristics</b>									
Total coliforms	NMP·(0.1 dm <sup>3</sup> ) <sup>-1</sup>	11	50	1 600	1 600	350	234	<b>640</b>	±753
Faecal coliforms	NMP·(0.1 dm <sup>3</sup> ) <sup>-1</sup>	6	0	600	4	50	4	<b>342</b>	±629

Explanations: BOD = biological oxygen demand, COD = chemical oxygen demand.

Source: own study.

are difficult to control in natural conditions, their management requires an efficient and versatile self-purification system that does not entail an aggressive intervention of the landscape environment in which they are located.

In all the samples, the pH values were close to the neutral range (7.0–7.5). These pH values favour the growth of a wide variety of microorganisms that support the biochemical processes of biodegradation. The presence of organic matter, nitrogen compounds and microorganisms of the coliform group is due usually to the anthropic action to which these aquariums are subjected, since human secretions, faecal or not, are potential sources of pathogenic microorganisms [CDC 2001; 2004; CRAUN et al. 1997; FIORILLO et al. 2001; HILDEBRAND et al. 1996; KRAMER et al. 1996; LEVY et al. 1998; SINCLAIR et al. 2009; TIRODIMOS et al. 2010; YODER et al. 2004]. Even when low concentrations of chemical contaminants are observed in the collected waters, they lack the microbiological quality required for bathing [NC-22 1999]. The removal of pathogenic microorganisms is one of the main objectives in the guidelines established by the World Health Organization (WHO). A sample of each of these selected water sources was used to feed the WA-VSSF bioreactors at laboratory scale to simulate the operation of the NSP treatment system.

**REMOVAL OF ORGANIC POLLUTANTS**

The efficiency in the elimination of organic pollutants can be seen in Table 3. The results of the ANOVA show that factors such as hydraulic loading and vegetation influenced the removal efficiency of COD, BOD, nitrates and ammonia. Significant differences were also detected among the water sources selected, as well as between the two samples, as expected.

Figure 2 shows the average concentrations of BOD, COD, nitrate and ammonium in the effluent of the bioreactors studied. The presence of vegetation (R1), in all cases,

**Table 3.** ANOVA for the removal efficiency of organic pollutants

Variability sources	Sum of squares (SS)	df	Middle squares (MS)	F-value	p-value
<b>BOD</b>					
A: H. loading rate	0.5055	1	0.5055	23.92	0.0000*
B: Vegetation	0.8070	1	0.8070	38.18	0.0000*
C: Water sources	68.3193	2	34.1597	1 616.13	0.0000*
D: Samples	4.5596	1	4.5596	215.72	0.0000*
AB	0.0431	1	0.0430	2.04	0.1628
AC	0.2781	2	0.1390	6.58	0.0039*
AD	0.0005	1	0.0005	0.02	0.8810
BC	0.0432	2	0.0216	1.02	0.3704
BD	0.0137	1	0.0136	0.65	0.4271
CD	60.6447	2	30.3224	1 434.58	0.0000*
Residual	0.6975	33	0.0211		
Total (corrected)	135.912	47			
<b>COD</b>					
A: H. loading rate	1.9012	1	1.9012	25.81	0.0000*
B: Vegetation	4.2346	1	4.2346	57.49	0.0000*
C: Water sources	588.965	2	294.4830	3 998.26	0.0000*
D: Samples	12.6557	1	12.6557	171.83	0.0000*
AB	0.0635	1	0.0635	0.86	0.3596

AC	1.0364	2	0.5182	7.04	0.0028*
AD	0.0022	1	0.0022	0.03	0.8647
BC	1.6084	2	0.8042	10.92	0.0002*
BD	2.5084	1	2.5084	34.06	0.0000*
CD	877.967	2	438.9840	5 960.18	0.0000*
Residual	2.4305	33	0.07365		
Total (corrected)	1 493.37	47			
<b>Nitrate</b>					
A: H. loading rate	0.0004	1	0.0004	16.39	0.0003*
B: Vegetation	0.0009	1	0.0009	32.13	0.0000*
C: Water sources	17.5684	2	8.7841	307 155.91	0.0000*
D: Samples	1.0591	1	1.0591	37 033.50	0.0000*
AB	0.0000	1	0.000002	0.07	0.7889
AC	0.0003	2	0.00015	5.46	0.0089*
AD	0.0000	1	0.00005	1.82	0.1864
BC	0.0004	2	0.0002	8.09	0.0014*
BD	0.0001	1	0.0001	3.57	0.0677**
CD	4.8881	2	2.4440	85 462.21	0.0000*
Residual	0.0009	33	0.00002		
Total (corrected)	23.5189	47			
<b>Ammonia</b>					
A: H. loading rate	0.0198	1	0.0198	25.35	0.0000*
B: Vegetation	0.0570	1	0.0570	73.03	0.0000*
C: Water sources	371.3220	2	185.6610	237 621.92	0.0000*
D: Samples	31.0972	1	31.0972	39 800.33	0.0000*
AB	0.0005	1	0.0005	0.64	0.4292
AC	0.0033	2	0.0016	2.11	0.1368
AD	0.0003	1	0.0003	0.49	0.4906
BC	0.0120	2	0.0060	7.68	0.0018*
BD	0.0024	1	0.0024	3.17	0.0840**
CD	89.4946	2	44.7473	57 270.74	0.0000*
Residual	0.0257	33	0.0008		
Total (corrected)	492.0350	47			
<b>Total coliforms</b>					
A: H. loading rate	184.08	1	184.08	21.14	0.0001*
B: Vegetation	0.75	1	0.75	0.09	0.7710
C: Water sources	1 729.50	2	864.75	99.29	0.0000*
D: Samples	2268.75	1	2 268.75	260.49	0.0000*
AB	0.75	1	0.75	0.09	0.7710
AC	282.16	2	141.08	16.20	0.0000*
AD	184.08	1	184.08	21.14	0.0001*
BC	1.50	2	0.75	0.09	0.9177
BD	0.75	1	0.75	0.09	0.7710
CD	1 729.50	2	864.75	99.29	0.0000*
Residual	287.41	33	8.71		
Total (corrected)	6 669.25	47			
<b>Faecal coliforms</b>					
A: H. loading rate	884.08	1	884.08	46.80	0.0000*
B: Vegetation	10.08	1	10.08	0.53	0.4702
C: Water sources	5 805.17	2	2 902.58	153.65	0.0000*
D: Samples	70.08	1	70.08	3.71	0.0627**
AB	6.75	1	6.75	0.36	0.5541
AC	452.16	2	226.08	11.97	0.0001*
AD	6.75	1	6.75	0.36	0.5541
BC	5.17	2	2.58	0.14	0.8727
BD	2.08	1	2.08	0.11	0.7419
CD	4 886.17	2	2 443.08	129.32	0.0000*
Residual	623.41	33	18.8914		
Total (corrected)	12 751.90	47			

Explanations: df = degrees of freedom, BOD = biological oxygen demand, COD = chemical oxygen demand, H. loading rate = hydraulic loading rate.

\* p-values < 0.05 were considered to be significant (95%).

\*\* p-values < 0.1 were considered to be significant (90%).

Source: own study.

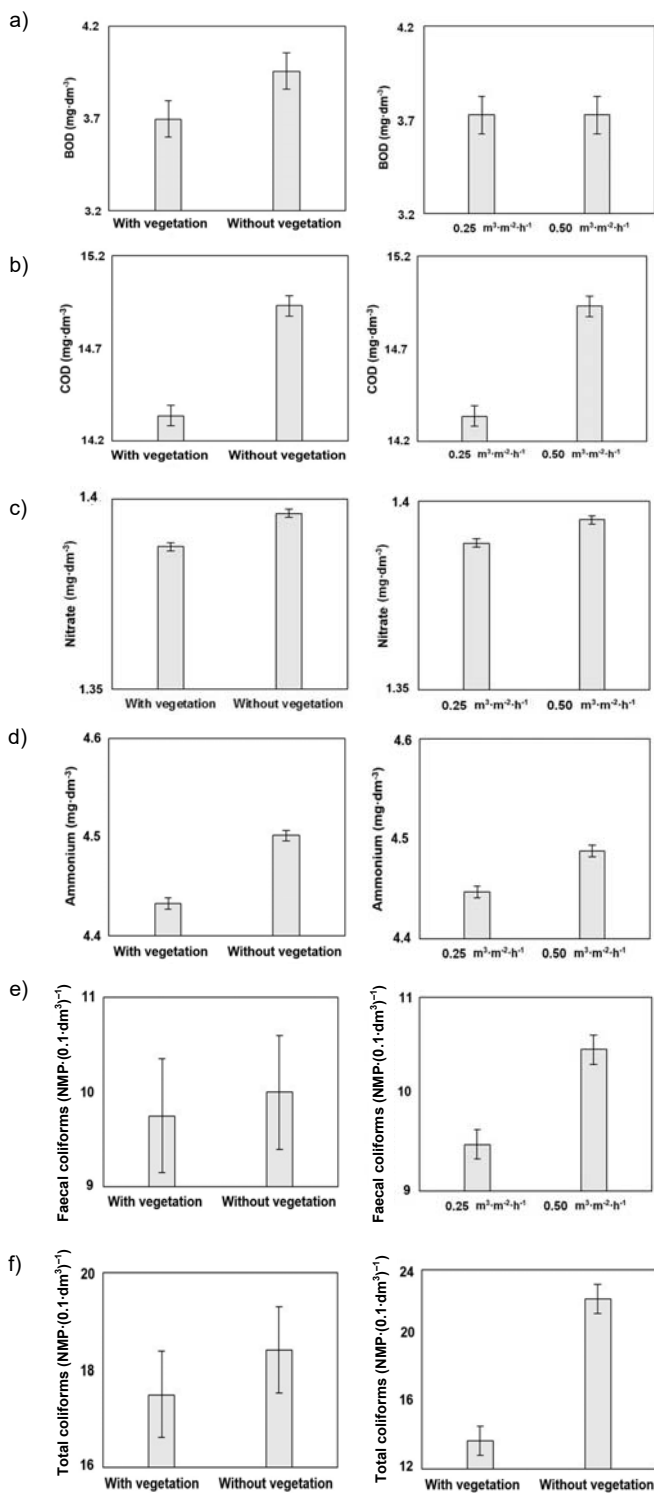


Fig. 2. Performance of the AW-VSSF bioreactors evaluated: a) BOD, b) COD, c) nitrate, d) ammonium, e) faecal coliforms, f) total coliforms; source: own study

favoured the reduction of nitrogen compounds (nitrates and ammonia) and organic materials (BOD and COD). This fact is explained by the presence of assimilation processes of organic compounds, or by the action of microorganisms associated with the rhizome of the *Typha* plants, which establish a favourable symbiotic mechanism for phytodepuration. The lowest concentration of ammonium obtained

in R2 reached a value of 2.15 mg·dm<sup>-3</sup>, which is within the limits allowed by sanitary regulations [NC-22 1999].

The elimination of COD was 4 times lower in R2 than in R1, with average values of 2.6% and 10%, respectively. Similarly, it can be seen in Figure 2 that the removal of pollutant compounds decreased in the following order: NH<sub>4</sub><sup>+</sup> 31% > NO<sub>3</sub><sup>-</sup> 20% and BOD 16% > COD > 10%. These figures suggest that the presence of oxidized nitrogen species limits the ability to remove contaminants unless phytodepuration is incorporated into the wetland as a treatment system. In comparison with the results obtained by GONZÁLEZ [2006], our findings suggest that the pollutant removal achieved by the AWs is less evident. Nevertheless, results similar to those obtained in this study were reported by KOUKIA *et al.* [2009], who assessed the operation of a combined AW-FWS and AW-SSF wetland system designed for rural domestic wastewaters treatment.

Increasing the applied hydraulic load, a significant reduction in COD was observed (Fig. 2b). For example, in the first week of evaluation of R1, and for a hydraulic load of 0.25 m<sup>3</sup>·m<sup>-2</sup>·h<sup>-1</sup>, an average concentration in the effluent of 3.55 mg BOD·dm<sup>-3</sup> was obtained, which implied a 25.7% of removal; and when the hydraulic load increased to 0.5 m<sup>3</sup>·m<sup>-2</sup>·h<sup>-1</sup>, the effluent had a concentration of 14.1 mg COD·dm<sup>-3</sup>, which denoted a removal of 16%. Both reactors exhibited low removal efficiency in comparison with other treatment systems; nevertheless, wetlands allow the treatment of waste water having pollutant concentrations less than 1.0 mg·dm<sup>-3</sup>, in spite of the limitations of these systems.

By increasing the applied hydraulic loads, a reduction in COD and BOD removal values was observed (Fig. 2a, b). However, R1 obtained better efficiency values compared with R2. In the first week of evaluation of R1, and for a hydraulic load of 0.25 m<sup>3</sup>·m<sup>-2</sup>·h<sup>-1</sup>, values of average BOD concentration in the effluent of 3.55 mg·dm<sup>-3</sup> (removal of 25.7%) were obtained. Also, by increasing the load to 0.5 m<sup>3</sup>·m<sup>-2</sup>·h<sup>-1</sup>, a COD removal of 16% was achieved, reaching concentrations in the effluent of 14.1 mg COD·dm<sup>-3</sup>. For both bioreactors, the removal efficiency was low compared with other treatment systems; however, wetlands allow the treatment of low-load wastewater, less than 1.0 g·dm<sup>-3</sup>, where some of these systems have their limitations.

The mechanism of ammonium removal in AWs has been previously described by AUSTIN [2006]. In the first stage, when the wetlands receive the water to be treated, ammonium cations (NH<sub>4</sub><sup>+</sup>) are retained on the negatively charged surfaces of the filter medium and then transported to the rhizomes of the plants. In the second stage, nitrate anions (NO<sub>3</sub><sup>-</sup>) are formed as products of ammonium oxidation, using the organic carbon of the feed water. Plants play an important role in all treatment processes with wetlands since they are useful for the absorption of nutrients and microbial adherence. In addition, nitrate elimination mechanisms in AWs are also related to the growth of plants and the nitrification process. Denitrification is the predominant mechanism in wetlands, especially when nitrate concentrations in wastewaters are high. This occurs in the anoxic sediment zones and in the anoxic microsites of

the biofilms adhered to the plants or the filter medium. For this reason, nitrate is one of the pollutants that is most efficiently removed using wetland systems [CUI *et al.* 2010]. Figure 2 shows the average concentrations of nitrate removal obtained in the effluent of the bioreactors studied. In R1, and for both hydraulic loads, nitrate concentrations were similar (average value of  $1.4 \text{ mg}\cdot\text{dm}^{-3}$ ). However, in the last week of evaluation, and for the lower hydraulic load applied, a lower value of nitrate removal was reached ( $0.915 \text{ mg}\cdot\text{dm}^{-3}$ ). Nitrogen is an essential nutrient for plants which remains in its organic form in the vegetation of AWs; although a portion of these nutrients is returned to the system when the plants die and decompose [DEBUSK 1999]. The biodegradation of chemical compounds often involves a series of biochemical reactions, and generally varies with the microorganisms present in the process [FAULWETTER *et al.* 2009; VYMAZAL 2007].

Control of the growth of *T. domingensis* was carried out in R1, where some parameters of plant growth were selected: rate of growth, number and width of the leaves, vegetation cover and wet weight of each emerged plant. Figure 3 shows the growth of *T. domingensis*. During the first week of system operation, plant sprouts emerged. After 30 days, the rate of growth of the plants was constant ( $0.24 \text{ m}\cdot\text{week}^{-1}$ ) and their height  $0.95 \text{ m}$ . At this time the first tiller emerged, the growth rate decreased to  $0.07 \text{ m}\cdot\text{week}^{-1}$ , the plants reached a length of  $1.60 \text{ m}$  and they had 12 leaves with an average width of  $0.011 \text{ m}$ . At the conclusion of the experiment, five leaves of each plant in R1 withered, but a density of 3 plants per rhizome was observed covering a surface area of  $0.025 \text{ m}^2$ . According to the criteria proposed by GONZÁLEZ [2000], the optimal coverage of AW-SSF reactors is reached when there are 4 plants per square meter of the wetland surface. Although the reactor had a high density of plants, no clogging of the filter was observed. The root system reached a depth of  $0.35 \text{ m}$ , and the wet weight of each plant was  $250\pm 25 \text{ g}$ .

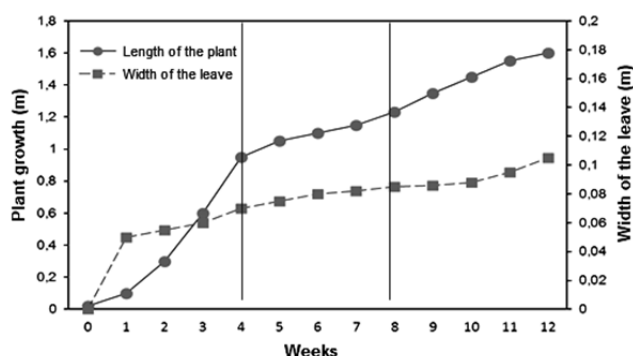


Fig. 3. Growth of *T. domingensis* in R1; source: own study

In order to study the vegetative behaviour of *T. domingensis* as plant material in AWs treating waters of NSPs, another bioreactor (R3) of larger scale ( $1.0 \text{ m}^2$ ) was used, but with similar characteristics of its filter medium to that of R1. A surface hydraulic load of  $0.25 \text{ m}^3\cdot\text{m}^{-2}\cdot\text{h}^{-1}$  was established and fed to R3 discontinuously during one year. The values of vegetation growth rate, number and width of leaves, vegetation cover and wet weight of each plant were

tabulated. Four rhizomes were planted in R3 and after the first 8 days of operation the buds began to sprout. Thirty days from plantation, the plants had a constant growth rate of  $0.35 \text{ m}\cdot\text{week}^{-1}$ , figure that is higher than that observed in R1. This growth behaviour was kept constant until day 105 from plantation. The parameters of vegetative growth during the first three months of operation of the reactors were very similar in R3 and R1. One year after the start-up of the bioreactors, the average height of the plants in R3 was higher than that of the R1 plants,  $1.65 \text{ m}$  and  $0.95 \text{ m}$ , respectively. Similarly, the total coverage observed in R3 was 20 plants per square meter; having an average of 16 leaves per plant with an average width  $0.015 \text{ m}$ . This coverage did not alter the water flow in the reactor. In R3 the wet weight of each plant was  $275\pm 21 \text{ g}$  and the root system had an average length of  $0.55 \text{ m}$ . These two last figures were higher than those observed in R1 plants.

With the objective of comparing the extent to which the *Typha domingensis* plants of the experiments had an adequate growth, *Typha sp.* plants from two different environments were selected: a natural wetland and an artificial wetland used for the treatment of domestic wastewater. In the former wetland, the average height of the plants was  $1.8 \text{ m}$ , value similar to that obtained in the R3 treatment. In the latter wetland the average height of the plants reached  $2.15 \text{ m}$ , their leaves had an average width of  $0.02 \text{ m}$ , and the average coverage density was 64 plants per square meter. These results indicate that the degree of contamination of a wetland can be estimated by simply observation of the number of plants present in it and what is their vegetative growth. *Thypha sp.* is an emergent aquatic plant that grows in tropical and temperate climatic regions, has a vigorous vegetative growth thanks to the presence of perennial rhizomes in its submerged stems [EID *et al.* 2012]. However, systematic pruning and the annual harvest are highly recommended.

## MICROBIOLOGICAL QUALITY OF TREATED WATER

The concentrations of faecal and total coliform in the treated wastewater can be seen in Figure 2e-f. The significant differences observed in the factors affecting to both variables (Tab. 3) determined the microorganism removal efficiency of the phytodepuration process. The presence of vegetation showed no significant differences in the removal of faecal and total coliforms in comparison with the removal obtained with no vegetation. Therefore, the microbiological quality of the water treated in the first system was attributed to mechanisms of adsorption-filtration and predation. These two mechanisms are present in slow sand filters used in the disinfection of water for human consumption and they are closely related to the design criteria of the two reactors used in our experiment. However, increasing the hydraulic load applied to both bioreactors resulted in a decrease of the removal efficiency (Fig. 2e-f). For example, the total coliform concentration of the effluent in R1, when the surface hydraulic load was  $0.25 \text{ m}^3\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ , was lower than 8 NMP per  $0.1 \text{ dm}^3$ , value that implies a removal efficiency of 92.5%, and the concentration of faecal coliforms was below 13 NMP per  $0.1 \text{ dm}^3$ , value that rep-



resents 90% of removal. The largest concentrations of faecal and total coliforms observed in the effluent of both bio-reactors were below the threshold of 100 NMP per 0.1 dm<sup>3</sup> established by the standard NC-22 [1999].

In a study conducted by CASANOVAS-MASSANA, BLANCH [2013], faecal microbial populations associated with four ecological pools were characterized. Three of the four pools evaluated exceeded the limits established for *E.coli* and enterococci, but the concentration of *Pseudomonas aeruginosa* and aerobic heterotrophic bacteria were in an acceptable range. They concluded his work affirming that the wildlife might be an important contributor of faecal contamination in NSPs. Some studies have shown that there is a significant tendency for clustering between *E. coli* and enterococci populations [MARION *et al.* 2010; WADE *et al.* 2006]. Likewise, studies on inland recreational waters have shown significant trends between increased gastrointestinal illnesses and *E. coli* or enterococci. In contrast, other authors have reported correlations between gastrointestinal illnesses and the number of swimmers, and no correlations between illnesses and faecal bacterial indicators, highlighting the importance of swimmer-to-swimmer transmission. Therefore, the relationship between health risks and faecal indicators concentrations is still under discussion [CASANOVAS-MASSANA, BLANCH 2013; COLFORD *et al.* 2007].

#### EVALUATION OF A FULL-SCALE NATURAL SWIMMING POOL

Once the laboratory experiments were completed, the operating conditions of a full-scale NSP swimming pool for recreational purposes were evaluated. The bathing zone had an area of 350 m<sup>2</sup> and an average depth of 0.7 m. The surface planned for phytodepuration occupied an area of 120 m<sup>2</sup>, planted all of it with *Typha domingensis* on a filter bed with the same operating characteristics as those of bio-reactors R1 and R3. Due to the time constraints to conclude this experiment, it was not possible to wait for the vegetation to develop completely, since the rhizomes of *Typha domingensis* had recently been planted. Therefore, the sanitary quality of the pool water was evaluated taking as reference one of the days with the largest number of visitors. The test was conducted in two sessions: during the morning opening hours, between 10:00 a.m. and 12:00 p.m., with a total of 250 bathers, and in the afternoon, between 2:00 p.m. and 5:00 pm, with the presence of 150 bathers (Tab. 4). In both bathing sessions, the values of some of the parameters that determine the quality of the water were measured, i.e.: pH, transparency, turbidity, residual free chlorine, nitrates, using a quick kit package (GPS Multi-Parameter Portable Water Quality Meter – HI 9829-00042).

In the morning session, the pH had an average value of 7.5, whereas in the late session the average value increased to a value of 7.74. These two pH values did not show any significant difference and were within the range considered acceptable (6.5–8.0). Similarly, both values were lower, and with a tendency towards neutrality, than the average value of 8.77 reported by GERALDES, BOAVIDA [2004], who assessed the growth rate of zooplankton and filamen-

**Table 4.** Monitoring of the control parameters to the full-scale NSP

Time	pH	Residual free chlorine (mg·dm <sup>-3</sup> )	Nitrates (mg·dm <sup>-3</sup> )	Ammonium (mg·dm <sup>-3</sup> )	Temperature (mg·dm <sup>-3</sup> )	Turbidity (mg·dm <sup>-3</sup> )	Transparency	Observation
10:00 a.m.	7	1.5	–	–	–	–	the coin at 1.0 m deep was observed	service opening with 250 bathers
11:00 a.m.	7.5	–	–	–	–	–	the coin at 1.0 m deep was observed	–
12:00 p.m.	8	1.5	–	–	–	–	the coin at 1.0 m deep was observed	service finished
2:00 p.m.	7.5	1	–	–	–	–	the coin at 1.0 m deep was observed	service opening with 150 bathers
3:00 p.m.	7.5	–	–	–	–	–	the visibility of the coin at 1.0 m decreased	–
4:00 p.m.	8	–	–	–	–	–	the visibility of the coin at 1.0 m decreased	–
5:00 p.m.	7.95	0.25	15.5	0.425	22.15	3.5	the visibility of the coin at 1.0 m decreased	service finished

Source: own study.

tous algae communities in three NSPs of Portugal. That latter pH figure represents a high alkaline media that promotes the growth of algae in aquatic systems.

The turbidity of the water of a swimming pool is attributed to the presence of dust, leaves and solid particles in suspension, or deposited in the bottom of the pooltub, that come from natural and anthropic actions. Other aspects such as wind, surrounding vegetation, addition of chemical compounds, rainfall, growth of microalgae and phytoplankton, and the bathers' own action, may also influence on the water turbidity. Cuban standards NC-22 [1999] and NC-441 [2006] establish that a swimming pool has adequate turbidity conditions if during its service the lowest pooltub sump, or a black circle of one centimetre in diameter, can be clearly observed from a depth of one meter. In the morning session the turbidity of the water satisfied the requisites of both standards, but at the end of the late session a decrease in the visibility of the pooltub sump was observed. This scenario was attributed to the incorporation of total and suspended solids by the anthropic action of the bathers, which, at this last stage, could not be completely removed by the phytodepuration system due to overload and lack of maturation of the wetland.

In the NSP swimming pool, the water used had an average concentration of free residual chlorine of 1.5 mg·dm<sup>-3</sup>, the right value of drinkable water, but lower than the concentration recommended for the disinfection of artificial pools. At the end of the afternoon session, the concentration of chlorine decreased to 0.25 mg·dm<sup>-3</sup>. This value was below the lower limit of the range established as permissible, 0.4–1.5 mg·dm<sup>-3</sup>. The decrease and degradation of free residual chlorine was produced by the increase in water temperature and the action of sunlight. These two circum-

stances were propitiated by the presence of a clear sky and an ambient temperature higher than 32°C.

At the end of the afternoon session the nitrate and ammonium average concentrations were 15.5 mg·dm<sup>-3</sup> and 0.45 mg·dm<sup>-3</sup>, respectively. The nitrate concentrations were within the allowed limits, but ammonium concentrations were slight above the allowed limits. The standard establishes that the nitrate and ammonium concentrations must not exceed the limit values of 20 mg·dm<sup>-3</sup> and 0.3 mg·dm<sup>-3</sup>, respectively. The elimination of nutrients in NSPs occurs in the filtration zone (artificial wetland), and in this experiment the latter had not reached optimum maturation. However, it was considered that the filtration zone could take on the nitrate and ammonium loads inherent to the use of the pool during a whole day. The values of nitrate and ammonium observed in our full-scale NSP were higher than those reported by GERALDES *et al.* [2014], 1.12 mg·dm<sup>-3</sup> and 0.04 mg·dm<sup>-3</sup>, respectively. These authors pointed out that the density of filamentous algae and phytoplankton decreased once the ecological balance was reached in the pools with well-established vegetation, as well as with the appearance of herbivore zooplankton.

Total and faecal coliforms concentrations determined at the end of the bath day evaluated remained below 200 NPM per 0.1 dm<sup>3</sup>, which is the limit established in the standard. These results indicate that, despite the systematic contributions of organic pollutants, the NSP maintained an acceptable level of safety. Likewise, the present study suggests that the phytodepuration carried out by an AW for the treatment of swimming pool waters achieved a satisfactory level of efficiency in the removal of physical, chemical and microbiological contaminants, allowing the parameters of water quality to maintain the appropriate values for a safe bath. This research has shown that NSPs are an adequate and sustainable alternative to diversify recreational options, promoting the elimination of the use of chemical compounds for water disinfection, as well as the increase in economic benefits and the improvement of social and environmental impacts.

An appropriate uniform distribution of the water to treat on the WAs, and fundamentally the AW-SSF, is important, which can be accomplished with distribution pipes and removable lids that can simplify the cleanliness works. Besides, operation of the system is transcendental that be accomplished at intervals, i.e., when the bath service in the NSPs is offered, or the previous tasks to service. An efficient artificial wetland system make possible rest periods in some zones of the filter medium, avoiding the clogging of the filtration zone due to the natural process of biological degradation and biofilms growth. Once that the filter medium of the AW is clogging, cannot be recovered without rest periods; several researches have showed that uniform rest periods recover the permeability of the wetland almost completely [VYMAZAL 2014].

## CONCLUSIONS

1. The findings of our work revealed that the removal of organic pollutants in the AWs of the NSPs was conditioned by the hydraulic load applied to the system and by

the presence or absence of vegetation. In the laboratory scale bioreactors, the presence of vegetation favoured the reduction of nitrogen compounds and organic materials. In addition, the purification capacity of both laboratory scale bioreactors allowed pollutant concentration values to be lower than the maximum values established in the standards that regulate the quality of bathing water in swimming pools.

2. *Typha domingensis* is an appropriate plant to use in the WAs that compose the phytodepuration system on the NSPs. A coverage density of 4 plants per square meter of the wetland surface is advisable, with annual periods of harvesting and systematic pruning to avoid the clogging of the filter medium.

3. In the phytodepuration area of the AW-VSSF, efficiencies of elimination of faecal and total coliforms were greater than 90%, and in the case of BOD the elimination efficiency reached 25.7%, but whenever the hydraulic load applied was less than 0.25 m<sup>3</sup>·m<sup>-2</sup>·h<sup>-1</sup>.

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### Wpływ obciążenia hydraulicznego i roślinności na fitoremediację w sztucznych mokradłach powiązanych z naturalnymi basenami pływackimi

#### STRESZCZENIE

Fitoremediacja jest szeroko stosowana do oczyszczania wody w rekreacyjnych i sportowych basenach pływackich. W pracy oceniono dwa sztuczne mokradła: jeden w rzeczywistej skali i drugi w skali laboratoryjnej, które obsadzono *Typha domingensis* w celu oczyszczania wody w basenie w klimatycznych warunkach Santiago de Cuba. Kiedy obciążenie hydrauliczne układu w skali rzeczywistej było mniejsze od  $0,25 \text{ m}^3 \cdot \text{m}^{-2} \cdot \text{d}^{-1}$ , stężenie zanieczyszczeń organicznych i mikrobiologicznych było mniejsze niż maksymalne limity wyznaczone przez kubańskie normy. W skali laboratoryjnej obecność roślinności sprzyjała eliminowaniu związków azotowych (azotanów i jonów amonowych) oraz materii organicznej (BZT i ChZT). Taki efekt tłumaczy się występowaniem procesów asymilacji związków organicznych lub aktywnością mikroorganizmów powiązanych z kłączami roślin, które tworzą symbiotyczny system korzystny dla oczyszczania. Minimalne stężenie jonów amonowych mierzone na odpływie z laboratoryjnego reaktora bez roślinności wynosiło  $2,15 \text{ mg} \cdot \text{dm}^{-3}$ ; mieściło się więc w granicach dopuszczalnych przez normy sanitarne.

**Słowa kluczowe:** fitoremediacja, naturalne baseny pływackie, sztuczne mokradła, *Typha domingensis*