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ENVIRONMENTAL AND SANITARY ENGINEERING

Treatment of landfill leachate by polyculture constructed wetlands planted with native plants

INGENIERÍA SANITARIA Y AMBIENTAL

Tratamiento de lixiviados de relleno sanitario por medio de humedales construidos sembrados con policultivos de plantas nativas

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Resumen

El uso de humedales construidos para el tratamiento de aguas residuales (doméstica, lluvia, lixiviados, drenaje de minas) ha venido incrementándose desde mediados de la década de los 90´s. Este trabajo presenta los resultados del estudio a escala piloto del desempeño de humedales construidos (CW) sembrados con policultivos de las especies tropicales Gynerium sagittatum (Gs), Colocasia esculenta (Ce) y Heliconia psittacorum (He) tratando lixiviado de relleno sanitario. Los CW fueron operados durante 7 meses a gravedad y flujo continuo (Q=0.5 m^3 d^{-1}). Tres CW fueron divididos en tres secciones y en cada sección (5.98 m^2) se sembraron 36 plántulas de una especie. La otra unidad se plantó aleatoriamente. La distribución final de plantas fue: : CW-I (He-Ce-Gs), CW-II (aleatorio), CW-III (Ce-Gs-He) and CW-IV (Gs-He-Ce). Todos los humedales recibieron el efluente de una laguna anaerobia de alta tasa (BLAAT®) que operó como tratamiento primario. Muestras de entrada y salida de cada CW fue analizada semanalmente para DQO total y filtrada y quincenalmente para metales pesados (Cd, Pb and Hg). Floración, longitud de tallo, clorofila y tasa fotosintética en las plantas fue medida quincenalmente. Las eficiencias de remoción fueron buenas; con mejor desempeño en el CW-IV (60-90%) para todos los parámetros, indicando que la distribución de las especies vegetales puede afectar la capacidad de remoción de los CW. Todas las especies vegetales presentaron buena respuesta fisiológica y crecimiento constante a lo largo de la investigación. Así, Las plantas nativas demostraron su capacidad para la fitorremediación de lixiviados de rellenos sanitarios y todas pueden ser categorizadas como acumuladoras de metales pesados.

Palabras clave: *Especies vegetales tropicales, heliconia psittacorum, humedal subsuperficial, lixiviados de relleno sanitario, remoción de metales pesados.*

Abstract

Use of constructed wetlands for wastewater treatment (domestic, storm water, landfill leachate, acid drainage mine) has increase since the mid 90´s. This paper presented the results of the study at pilot scale of constructed wetland (CW) for landfill leachate (LL) treatment planted with polycultures of tropical species Gynerium sagittatum (Gs), Colocasia esculenta (Ce) and Heliconia psittacorum (He). The CW cells were operated during 7 months at continuous gravity flow (Q=0.5 m^3 d^{-1}). Three CWs were divided into three sections, and each section (5.98 m^2) was seeded with 36 cuttings of each species. The other unit was planted randomly. The final distribution of plants in the CW cells was: CW-I (He-Ce-Gs), CW-II (randomly), CW-III (Ce-Gs-He) and CW-IV (Gs-He-Ce). All CW cells received pre-treated LL from a high-rate anaerobic pond (BLAAT®) operating as primary treatment. Influent and effluent from each CW cell were analysed for total and filtered COD weekly and heavy metal (HM) (Cd, Pb and Hg) fortnightly. Flowering, stem length, Chlorophyll and photosynthetic rates in plants were measured fortnightly. The removal efficiencies were good; with better performances in CW-IV (60-90%) for all parameters, indicating that plant distribution may affect the removal capacity of CW cells. All plants presented a good physiological response and constant growth along the research period. The native plants thus demonstrated their suitability for phytoremediation of LL and all could be categorized as HM accumulators.

Keywords: *Heliconia psittacorum, heavy metal removal, landfill leachate, subsurface wetland, tropical plants.*

1. Introduction

Nowadays sanitary landfill is the most used method of solid waste disposal in the world. Landfill Leachate (LL) is a high and complex polluted residual liquid that results from rainwater infiltration through buried solid waste, assisted by biochemical processes inside the landfill and water content from waste itself (Renou et al., 2008). LL is recognized as a high-strength wastewater, characterized by a high Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD), high concentration of inorganic salts, heavy metals (HM) and potential toxicity (Žgajnar et al., 2009). Landfill leachate complexity engenders special requirements for its collection, storage, treatment and disposal.

A high content of heavy metals may be found on municipal Landfill leachate, usually when domestic waster is mixed together with industrial waste or sludge, or due to the lack of separation of hazardous materials from domestic waste, combining all sorts of residues. Anthropogenic activities contribute to release HM into the environment. Pb, Cd and Hg are present simultaneously in the environment by human activities and LL is certainly one of the major sources.

Constructed wetlands have been used successfully for removal of heavy metals (HM) present in Landfill leachate and wastewaters and are classified among the biological methods that use phytoremediation for polluted liquid treatment. In this sense constructed wetlands as a phytoremediation technique, has become an attractive and feasible technology for liquid waste management, given its many advantages such as low energy consumption, low cost implementation and operation, high efficiency for pollutant removal and aesthetically characteristics which enables it with a valuable social acceptance (Akinbile et al., 2012).

Plants are widely used for the removal of pollutants from polluted matrixes (water and soil), with an important experimental and practical approach over the last decades. Plant species play a crucial role in pollutant removal from Landfill leachate through absorption, cation exchange, filtration and chemical changes through root; also, by providing specific micro-environments inside the Constructed Wetlands that enhance its performance. There are large number of studies that shows that plant species can accumulate heavy metals in their above and underground tissues (*i,e, Typh alatifolia* and *Cyperus malaccensis*). Therefore, plant species selection is a very important step for implementing Constructed Wetlands phytoremediation, which is often done by considering previous applications and research.

In this sense, the selection of the plant species is an important step in CW design because they should survive the potential harmful effects of the influent and its variable loading rate. The most widely used CW design in Europe is the horizontal subsurface flow system vegetated with the common reed (*Phragmites australis*), although other plant species, such as cattails (*Typha* spp), bulrushes (*Scirpus* spp.), and reed canary grass (*Phalaris arundinacea*), have been used for both domestic and industrial wastewater treatments (Calheiros et al., 2007).

According to Maine et al. (2009), constructed wetlands allow good retention of heavy metals and several studies from authors world-wide have shown the capacity of macrophytes for accumulation and sequestration of metals in shoot and root plant parts in non-metabolic-active tissues in less harmful forms (Kupper et al., 2007). However, more researches are needed in order to find new native or indigenous tropical species due to the role that the plant species play in the phytoremediation process.

In this paper, we focused on the performance of Horizontal CW at pilot scale planted with polycultures of the tropical plants *Gynerium sagittatum* (Gs), *Colocasia esculenta* (Ce) and *Heliconia psittacorum* (He) treating LL under tropical conditions. In addition, the growth and physiological response of the plants growing in the CW treating LL were evaluated.

2. Materials and methods

2.1 Experimental set-up

The experiment was carried out during 29 weeks in the Presidente regional landfill (3^{o} 56`01.54”

N and 76º 26`26.05”O) at the San Pedro village (southwest Colombia). Four rectangular sub-surface CW were constructed in concrete (7.80 x 2.30 x 0.60 m in length, width and depth, respectively) and run in parallel. Each tank was filled to a depth of 0.50 m with gravel (diameter ϕ= 25 mm and porosity η = 40%).

Three CW cells were divided into three equal sections, each one 2.6 x 2.3 m in length and width, respectively. At each section, 36 healthy-looking cuttings (0.10-0.15 m height) of one single species (Gs, Ce or He) were placed in a chosen order. Meanwhile, in the other CW cell, 36 cuttings of each species were planted randomly throughout the whole length. The plant density was 6 cuttings m^{-2}. The final distributions of plant species in CW cells were: CW-I (*He-Ce-Gs*), CW-II (randomly), CW-III (*Ce-Gs-He*) and CW-IV (*Gs-He-Ce*). The CW cells were fed daily by gravity under continuous regime with a water inflow of 0.5 m^3 d^{-1} each, and the theoretical HRT of 7 d. All bioreactors received pre-treated LL from a high-rate anaerobic pond (BLAAT®), that is recent technology that combines the high removal efficiency of a UASB reactor with the construction and functional simplicity of an anaerobic pond (Peña et al., 2003)

The experimental design was blocks since it was not possible to have replicates. The main factor was constructed wetland type (I, II, III and IV) and the block factor corresponded to the timing of the measurements. The Friedman non-parametric statistical test was used as alternative option since this test is one of the best for the random block design. The Post Hoc test was used in order to know the difference between the CW cells.

2.2 Sampling and chemical analyses

Influent and effluent of each CW cell was analysed weekly for total and filtered COD (CODf), and fortnightly for Hg, Cd and Pb. All of the parameters were determined according to APHA-AWWA (2005). Likewise, temperature (T), pH, redox potential (Eh), electric conductivity (EC) and dissolve oxygen (DO) were measured five times a week using a portable VWR symphony SP90M5 meter (OpticsPlanet, USA).

In plants, leaf chlorophyll content was measured fortnightly in three leafs in nine plants (three of each species) in each CW cell using a Minolta (SPAD-502) chlorophyll-meter. The photosynthetic rate was measured in two leafs of two plants (one of each species) in each CW cell using a CI-340 Ultra-Light Portable Photosynthesis System. Stem length and flowering were measured in three plants of each section in each CW cell.

2.3 Heavy metal determination in plant tissue and landfill leachate

At the end of the experiment, all plants were harvested, washed thoroughly with tap water, and rinsed with deionized water. The whole plants were weighted using a digital scale (FENIX, LEXUS electronics scale, ± 0.1 g). Then, the plants were divided into above and below ground tissue and weighted again. Subsequently, the samples were dried at 80 °C for 24 hours (MLW Warmeschrank WS oven) and were ground with a crushing machine (IKA 14 basic, A11-2 blades). Approximately, 0.5 g of sample material was digested in 10 ml of HNO_3 (65%) and underwent microwave digestion (CEM-Mars 5 X-press Duotemp, version 194A07). Cd and Pb concentrations present in the samples were determined by an ICP-MS (Inductively Coupled Plasma - Mass Spectrometer; Thermo scientific Type: X-series 2, ± 1 μg L^{-1}). Hg was measured by cold vapour atomic absorption spectrometry (Shimadzu AA 6300, ± 1 μg L^{-1}) with a graphite furnace (Shimadzu HVG-1). More details of the analytical determination are described in Madera et al. (2015).

For landfill leachate, fortnightly 220 ml influent and effluent of each CW cell were grabbed for HM determination. 50 ml of water sample was mixed with 0.5 ml of HNO_3 (65%) and then the HM was determined by the same analytical methods used for the plant samples.

2.4 Bioconcentration and translocation factors

The Bioconcentration Factor (BCF, L kg^{-1}) was calculated as follows (Soda et al., 2012):

$$BCF = C_P/C_W \quad (1)$$

Where CP is the metal concentration in the whole plant tissue (mg kg^{-1}-DW) and CW is the metal concentration in the water (landfill leachate) (mg L^{-1}).

The translocation factor (TF) was calculated as follows (Soda et al., 2012):

$$TF = CA/Cu \quad (2)$$

Therein, C_A is the metal concentration in above ground tissues (mg kg^{-1}-DW) and C_u is the metal concentration in underground tissue (mg kg^{-1}-DW).

The Relative Growth Rate (RGR) per plant and per period was calculated using Eq. 3.

$$RGR = \text{Ln } SL_2 - \text{Ln } SL_1/(T_2 - T_1) \quad (3)$$

Where SL_1 and SL_2 are estimates of the stem length for times T_1 (beginning of period) and T_2 (end of period), respectively (Leopold & Kriedemann, 1975).

3. Results and discussion

3.1 Physicochemical characteristics

The influent LL (Table 1) to the CW cells is within the classification range as an intermediate LL (Renou et al., 2008). pH was alkaline for both influent and effluent (Table 1), with values varied between 5.8 to 9.5 and 6.8 to 9.3, respectively and no significant differences between bioreactors were observed. The temperature fluctuated between 26 and 27 ^{o}C in all constructed wetlands units for both inlet and outlet. DO showed in all constructed wetlands slight variation between outlet and inlet, being major in the effluent (2.3 mg L^{-1}), indicating a potential oxygen translocation in the rhizosphere by the plant species. Respect to EC, no differences between experimental units was detected. Water column of the CW cells achieved a little anoxic environment seven days after having started operation the experimental units, since Eh values along the studied period were negatives varied between -22 and -6 mV.

Table 1. *Influent and Effluent LL quality in the CW cells along the study period.*

Parameter	n	Influent	Effluent CW-I	Effluent CW-II	Effluent CW-III	Effluent CW-IV	Guidelines Colombia[1]	Guidelines FAO[2]
		Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD		
Flow (m^3 d^{-1})	29	0.5±0.1	0.4±0.1	0.4±0.1	0.4±0.1	0.3±0.2	-	-
pH (Und)	59	5.8-9.5	6.8-9.2	7.1-9.3	7.1-9.3	7.0-9.3	6 - 9	6.5-8.4
T (^{o}C)	61	27.3±1.6	26.4±1.7	26.4±1.6	26.7±1.6	26.5±1.6	≤40	
EC (mS cm^{-1})	58	4.8±1.1	3.7±0.9	3.8±0.9	3.7±0.9	3.4±1.1	1.5	0.7-3
DO (mg L^{-1})	37	0.8±1	1.7±2.3	1.1±0.9	2.2±2.9	2.3±1	4.0-5	-
Eh (mV)	32	-52.9±140.4	-89.5±109.6	-75.1±102.4	-64.9±101.5	-63.9±109.5	-	-
COD total (mg L^{-1})	29	627.6±179.3	388.3±142.5	421.3±152.7	405.3±152.4	378.1±152,1	2000	-
COD filtered (mg L^{-1})	29	463.7±169.2	285.4±141.6	307.2±154.9	299.1±150.1	265.4±119.3	-	-
Hg (µg L^{-1})	12	3.6±6.1	3.4 ±5.3	2.0±2.4	1.8±1.6	1.8±1.9	10	-
Pb (µg L^{-1})	12	3.9±3.5	2.3±1.2	3.3±2.6	3.2±2.9	3.8±3.4	200	-
Cd (µg L^{-1})	12	1±1.5	0.3±0.2	0.3±0.3	0.4±0.4	0.2±0.2	50	-

[1] Ministerio del Ambiente y Desarrollo Sostenible, Resolución 0631/ 2015 (Article 14).[2]. FAO (1985).

3.2 Organic matter removal

The experimental CW cells showed a good organic matter (COD & COD_f) removal capacity, where CW-III was the unit with best functioning for COD_f (67%) and CW-IV for total COD (50%). However, no significant difference was found between the CW cells ($p>0.05$) for COD_f, but for total COD the situation was opposite being CW-IV a wetland cell with better performance. These results indicate a potential effect of the plant species distribution on the performance of the bioreactors for organic matter removal.

3.3 Heavy metal removal

All heavy metal (Pb, Cd & Hg) concentrations in the effluent were more than three times lower than the Colombian guidelines (Table 1). The removal efficiency of this HM varied between 10 to 80%. The removal of Pb was poor in all CW cells (Table 1). The CW cells showed good Cd removal with CW-IV being the best Cd removing CW cell (80%).

3.4 Bioconcentration and translocation factors in the native plants

The *Gs* was the plant with higher HM accumulation in the tissues in all CW cells (Figure 1). Gs presented a higher BCF for Cd (84 Lkg^{-1}). The BCF of Hg and Pb fluctuated between 0.2 and 4.5 L kg^{-1}. Ce presented a TF < 1 for all HM, the other two plant species have a TF>1 for Pb and Hg. He had a TF > 1 for Cd in CW-II and CW-IV.

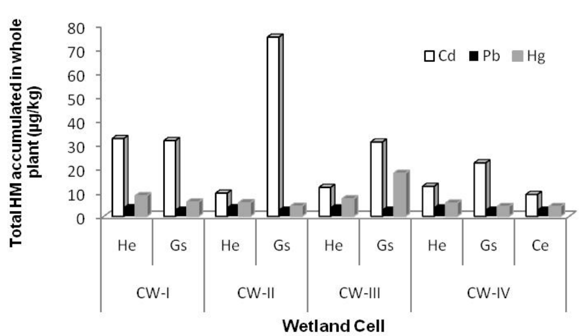

Figure 1. *Heavy metal accumulation by plant in each CW cells.*

3.5 Growth and physiologic response

Ce presented a higher chlorophyll-a content (81.1 SPAD) in CW-IV. *He* and *Gs* reached major Chlorophyll-*a* values in CW-II with 77.9 and 67.6 SPAD, respectively (Figure 2). The Chlorophyll-*a* content increases along the research period. The photosynthetic rate (PR) in *Gs* varied between 9.7 and 10.7 µmol m^{-2} s^{-1}. In *Ce*, the photosynthetic rate does not change between CW cells with average values of 5.8 µmol m^{-2} s^{-1}. The growth behaviour was good. *Heliconia sp* had a relative growth rate (RGR) between 0.0003 a 0.0093 cm d^{-1}. *Ce* was good in CW-III and CW-IV with values between 0.0197 and 0.0231 cm d^{-1}. *Gs* showed the higher RGR with an average value of 0.0214 cm d^{-1} in all CW cells. *Heliconia sp* flowered in 60% of the planted cuttings; only 10% of the *Gs* species developed flowers in two different periods: in the beginning and at the end of the research period. Between 10 to 20% of the *Ce* plants presented flowers, and only in CW-IV.

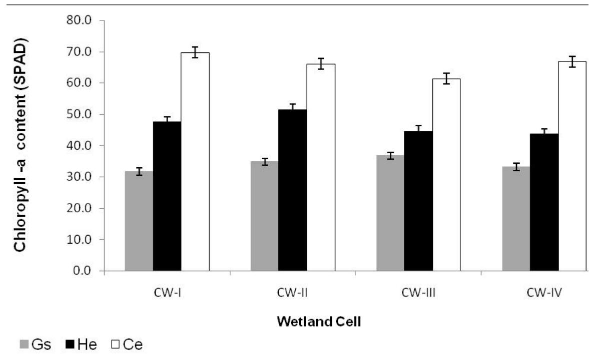

Figure 2. *Average Chlorophyll-a content in the native plant species used in the CW cells.*

4. Discussion

4.1 COD removal by the CW cells

The obtained data showed that CW planted with Polyculture of the tropical studied plant species has a higher potential to use as a technology for LL treatment. COD_f removal efficiencies in the all CW cells were good being CW-III the best unit (67%). In the case of total COD removal, CW-IV was the better CW cell with an efficiency of 50%. The performance of the wetland cells can be described as a satisfactory if consider that the influent of the units was intermediate LL, which special characteristic is lower biodegradable organic matter content and higher amount of refractory compounds. An effluent from all CW cells meets the Colombian legislation for total COD (Table 1). The results of the present study were better that the data reported Turker et al. (2013), who evaluated Horizontal CW cells planted with Polyculture of the species *Phragmities a* y *Typha l*, treating acid mine drainage, who obtained a total COD removal of 35%. Likewise, the results were better that data described by Bulc (2006) who worked with LL. Similarly, the data of the present study were better than obtained results by Yalcuk & Ugurlu (2009), who evaluated 3 pilot-scale Constructed Wetlands, two with vertical flow (VF1 and VF2) and one with horizontal flow for Landfill leachate treatment. All three wetland cells were planted with *Typha latifolia* and the obtained average removal efficiency was above 30% for COD, where horizontal wetland cell was more effective in COD removal.

According with Sttotmeister et al. (2006), removal of organic matter in constructed wetlands occurred under aerobic, anaerobic or anoxic environment by microbiological transformation due to higher activity of microorganisms that are present in the biofilm that is formed in the surface of the gravel media and plant roots. Regarding with the OD and Eh values in the present study, big proportion of the organic matter was removed mainly under anoxic and anaerobic conditions together with some type of microaerophilic metabolism near to the roots and the surface of the gravel bed induced by oxygen translocation by plant species in the rhizosphere.

Plant affect on the Eh conditions in the water was demonstrated by Białowiec et al. (2012), who worked with CW at microcosms scale planted with reed and willow treating Landfill leachate. They found that plant has an effect on redox potential levels in water column, registering anoxic conditions in the reed wetland cell. In the other wetland cell, Redox potential fluctuated significantly in the rhizosphere, mainly in light hours, with higher declines in the morning. This points out that redox potential an indicator if the type of activity is anoxic, aerobic or anaerobic within the Constructed Wetlands.

4.2 Heavy metal removal

The heavy metal removal efficiencies of all constructed wetlands cells varied between 10 and 80%. Furthermore, the concentration in the effluent of the CW cells was below of Colombian standards (Table 1) for the studied HM. Phytoaccumulation, precipitation with insoluble salts, plant uptake probably took place inside the CW cells as mechanisms for HM removal.

The evaluated native emergent species could tolerate and accumulate heavy metals (Figure 1) and still showed healthy growth while treating LL containing a multiple heavy metal cocktail and toxic organic compounds. This feature is similar to previous reports with emergent plants employed in CWs such as *Phragmites australis, Arabis paniculata, Brasica juncea, Thalium geniculata, Typha latifolia*, and *Colocasia esculenta* (Skinner et al., 2007; Bindu et al., 2008; Soda et al., 2012; Anning et al., 2013).

4.3 Bioconcentration and translocation factor

The BCF of Pb was 230 times lower than the value found by Soda et al. (2012), who worked with CW planted with *Acorus gramineus* and *Cyperus alternifolius* L. Meanwhile, the BCF of Hg was 10 times higher that the value reported by Skinner et al. (2007) who worked with four plant species, including *Colocasia esculenta.* This confirms that different plant species develop diverse mechanisms to accumulate and tolerate heavy metal, most certainly depending on the specific environmental conditions.

The *Gs* and *He* presented a TF> 1 for Hg, except in CW-III and Ce where the plant had a TF < 1. These results indicate that the Hg accumulation in the shoots compared to those in the roots were higher by at least one order of magnitude in all plant species.

Regarding Pb, all plants presented a TF > 1, except for *Ce* in CW-IV. The TF of Cd was > 1 only for *Heliconia sp* in CW-II and CW-IV. The other species showed low mobility of this metallic ion to aerial tissues. Cd or Pb induces numerous harmful effects in the biochemical machinery required for cell survival. Both Cd and Pb have many action sites within the plant. It is more probable that accumulation of these metals is associated to a sequestration mechanism in a less toxic form. In our study, the three native species evaluated accumulated more Cd than Pb (Figure 1), thus suggesting a plant strategy to avoid the toxic effects of Pb. These conditions of metal accumulation in aerial tissue can contribute to the removal of the HM from the systems through harvesting the shoot tissue.

4.4 Physiological and growth plant species response

The *Gs* was the plant species with a lower Chlorophyll-*a* content and was similar in all CW cells (Figure 2). The Chlorophyll-*a* content in *Ce* was high, reaching the highest values in CW-IV and CW-I. *Heliconia sp* presented medium Chlorophyll-*a* values and was similar for all CW cells (Figure 2). This situation indicates than those species developed a tolerance capacity to LL and plant distribution inside the CW cells could have an effect on the physiological response of the plant species. According with Sims & Gamon (2002), Chlorophyll-*a* content in the leaf is a good indicator for estimation the response of the plant species to environmental stress.

Gs was the species who presented higher photosynthetic rate values, surprisingly *Gs* was the plant with the lower Chlorophyll-*a* content, indicating a very good adaptation of the physiological apparatus of the plant to the environment. The photosynthetic rate in *Gs* was higher than *Ce* and this condition could be related with the plant metabolism for carbon fixation of the plant species, since *Ce* is a C-3 plant, which characteristic is a lower photosynthetic activity compared to C-4 plants that is the group of *Gs*. Likewise, the photosynthetic rate could reflect the state of the physiological apparatus of the plant species (Huang et al., 2010).

Heliconia-He reached stem lengths between 0.5 to 0.6 m, considered as a normal range for this species (0.5 to 1.5 m, Herbario Universidad de Antioquia, 2008). *Gs* presented the higher RGR (0.0214 cm d^{-1}) and reached a stem length of 2.5 m, a value considered good, since it is in the normal range of this species (2.4-5.0 m, Herbario Universidad de Antioquia, 2008). *Ce* showed a constant RGR in CW-III and CW-IV. Likewise, the stem length was between 0.7 and 1.4 m, slightly higher than the normal growth for this species (0.7-1.2 m, Herbario Universidad de Antioquia, 2008).

Cheng et al. (2009) suggested that the effect of plant growth in the removal of pollutants in CW is directly, since plants with higher RGR presented higher contaminants removal rates. In the present study, *Gs* and *He* accumulated higher HM concentrations in their tissues without affecting their physiological and growth apparatus. This lower effect of the LL on the growth and physiological machinery of the studied plant species can be ratified in the flowering process of the plants, since all species flowered along the study period. This could be due a plant response to the stress conditions than the plants were exposed. Parvaez et al. (2011) reported that plants exposed to stress can flower in order to produce the next generation acclimated to the stress condition.

5. Conclusions

The horizontal flow constructed wetlands effectively removed COD, COD_f and heavy metal (Pb, Cd, Hg) from pre-treated landfill leachate and achieved concentrations below the Colombian standard. Hence, development of this types of constructed wetlands at full-scale is an attractive technology for landfill leachate treatment in countries with low resources and high necessities to protect the environment and public health.

The obtained bioconcentration of HM was Cd>Hg> Pb, indicating a higher affinity of the species to remove Cd. The plant species did not

not reach the HM accumulation as hyperaccumulators, but they can be considered as HM accumulators. G. *sagittatum* was the species with the best performance, followed by H. *psittacorum* and C. *esculenta.*

The evaluated plant species maintained a good growth with average values above the theoretical value for the species and physiological response with good Chlorophyll-*a* content and higher photosynthetic rate along the study, indicating a minimum effect of the stressed environment on the plant species response. The physiological and growth apparatus were not damaged and all species flowered along the research period.

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