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Removal of Glyphosate in Agricultural Runoff Using Subsurface Constructed Wetlands in Monocultures and Polycultures of Tropical Plants

Graciano Aguilar-Cortés [1], Gustavo Martínez-Castellanos [1,2,*], Georgina Martínez-Reséndiz [3,4], Sergio Aurelio Zamora-Castro [5], María Cecilia Monroy-Pineda [6] and Luis Carlos Sandoval Herazo [4,6,*]

1 Division of Graduate Studies and Research, Tecnológico Nacional de México/Instituto Tecnológico Superior de Misantla, Km 1.8 Carretera a Loma del Cojolite, Misantla 93821, Veracruz, Mexico
2 Biochemical Engineering Department, Misantla Institute of Technology, Misantla 93821, Veracruz, Mexico
3 CONACYT (Consejo Nacional de Ciencia y Tecnología) in Tecnológico Nacional de México Campus Misantla, Misantla 93821, Veracruz, Mexico
4 Wetlands and Environmental Sustainability Laboratory, Division of Graduate Studies and Research, Tecnológico Nacional de México/Instituto Tecnológico Superior de Misantla, Km 1.8 Carretera a Loma del Cojolite, Misantla 93821, Veracruz, Mexico
5 Facultad de Ingeniería, Construcción y Hábitat, Universidad Veracruzana, Bv. Adolfo Ruíz Cortines 455, Costa Verde, Boca del Rio 94294, Veracruz, Mexico
6 Facultad de Ingeniería, Universidad de Sucre, Cra. 28 #5-267, Puerta Roja, Sincelejo 700003, Sucre, Colombia
* Correspondence: gmartinezc@itsm.edu.mx (G.M.-C.); lcsandovalh@gmail.com (L.C.S.H.)

Abstract: Glyphosate (GLY) is the most widely used herbicide in agriculture worldwide, posing a significant contamination risk to rivers, lakes, wetlands, and soils. Its ultimate fate represents a potential threat to the health of both terrestrial and aquatic ecosystems. This study evaluated the removal efficiency of glyphosate and conventional pollutants in mesocosm-scale horizontal subsurface flow-constructed wetlands planted with *Canna indica*, *Heliconia psittacorum*, and *Alpinia purpurata* in runoff water contaminated with glyphosate. Additionally, the study examined the performances of these species in monoculture and polyculture settings of tropical ornamental plants. *Canna indica* exhibited the highest growth (up to 160 cm) in both monoculture and polyculture conditions, as well as the highest removal efficiencies for total nitrogen (TN), total phosphorus (TP), and phosphate (PO_4^{3-}), achieving a 91%, 93%, and 98% removal, respectively. Polyculture systems demonstrated a superior ammonium removal efficiency, reaching 94%. *Alpinia purpurata* (>5 ppm after 40 days) and *Heliconia psittacorum* (>5 ppm after 200 days) were the most effective species for glyphosate removal. Glyphosate can be effectively removed from aquatic environments through constructed wetlands planted with ornamental species, offering a sustainable approach to mitigating herbicide contamination in water bodies.

Keywords: glyphosate; constructed wetlands; ornamental plants; agricultural runoff

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1. Introduction

n-(phosphonomethyl)glycine, commonly known as glyphosate (GLY), is the most widely used herbicide globally [1] For many years, GLY was considered to be an inert compound [2] however, due to its ubiquity in the environment, it has been detected in various organisms and even in surface waters [3]. Concentrations of up to 0.442 ppm have been identified in drinking and surface water [4], 5200 ppm in runoff water [5], and 5800 ppm in urban streams [6]. Moreover, it has been shown that GLY can negatively impact the chemical and biological processes of soil [7], where levels of up to 5500 ppm have been

found [8]. Additionally, concentrations of up to 9 ng/m^3 have been recorded in the air [9]. It has even been detected in human semen, with a concentration of 0.19 ± 0.23 ng mL^{-1} [10].

In 2015, the World Health Organization (WHO) reclassified glyphosate as a probable carcinogen. Regarding mammalian toxicity, its acute LD_{50} (lethal dose for 50% of individuals) is approximately 5037 mg kg^{-1} [11]. Regulatory agencies set specific limits for glyphosate presence in various media, such as drinking water and food, to protect public health. The U.S. Environmental Protection Agency (EPA) has established a Maximum Contaminant Level (MCL) for glyphosate in wheat grains at 5 parts per million (ppm), wheat forage at 100 ppm, sorghum grains at 15 ppm, oats at 20 ppm, and drinking water at 0.7 mg L^{-1}. This compound, like other pesticides, eventually reaches both surface and groundwater bodies, including lakes and oceans, through various mechanisms [12,13]. Water contamination is primarily the result of agricultural and urban runoff, where herbicides/pesticides enter via soil leaching or the direct discharge of contaminated wastewater [14,15]. Due to its potential health risks, efforts are being made to eliminate this compound from environmental compartments, particularly water.

The removal of GLY from aquatic systems has been extensively studied, particularly concerning the treatment of industrial effluents [16], as well as water purification in urban environments, rivers, and lakes [17,18]. A variety of methods have been explored for its remediation, which primarily include physical processes such as adsorption [19], membrane filtration, and conventional filtration; chemical processes, including coagulation, flocculation, TiO_2 photocatalysis [20,21], precipitation coupled with advanced oxidation processes [22], and the heterogeneous Fenton process utilizing zero-valent iron [23,24]; and biological approaches such as bioremediation via microbial degradation [25–27] and, in certain instances, nature-based technologies.

Nature-based technologies, such as constructed wetlands [28], are gaining increasing attention due to their advantages, including low energy consumption, simplicity in operation and maintenance, and effective purification performance through physical, chemical, and biological processes [29,30]. Constructed wetlands can be classified into different types based on their design and water flow. A prominent example is horizontal subsurface flow wetlands (HSFWs), where wastewater moves horizontally from the inlet to the outlet, passing through a porous bed in which macrophytes establish roots that play a key role in the removal of contaminants [31]. In terms of design, mesocosm-scale wetlands are systems that offer a significant advantage by allowing for the reproduction of controlled experimental units that simulate real-field conditions. This capability facilitates a detailed analysis of the factors influencing the efficiency of contaminant treatment, thereby optimizing the design and performance of these systems [32].

An additional important factor in constructed wetlands is the role of plants, which are recognized as essential for nutrient removal. Given their importance, the selection and distribution of plant species, whether in monoculture (using a single species) or polyculture (using multiple species), are pivotal. To date, most studies have focused on monocultures of various plant species [33]. However, the use of polycultures can not only enhance the functional diversity of a system, but also improve its aesthetic value and optimize its root biomass distribution, thereby facilitating the establishment of habitats for microorganisms [34].

This study focuses on evaluating the efficiency of subsurface constructed wetlands at the mesocosm scale for the removal of glyphosate and conventional contaminants from agricultural runoff. These systems have demonstrated themselves as a sustainable and cost-effective alternative for treating contaminated water, providing purification processes based on physical, chemical, and biological mechanisms. Given the widespread use of pesticides in agriculture, especially in Mexico's primary agricultural states, such as Sinaloa,

Chiapas, Colima, Jalisco, Nayarit, Sonora, Tamaulipas, and Veracruz [35], this study aims to contribute to the understanding of constructed wetlands in the treatment of agricultural pollutants. The research will provide valuable insights for optimizing these systems in regions with intensive pesticide use. Based on the obtained results, the study seeks to establish scientific foundations for the design and implementation of sustainable strategies to mitigate water pollution in agricultural areas, promoting the use of natural and accessible technologies for treating contaminated runoff, with the goal of scaling this process in the short term.

2. Materials and Methods

2.1. Study Area

This study was conducted at the Tecnológico Nacional de México (TecNM), Misantla Campus, Veracruz, Mexico, from June 2022 to April 2023. The climatic conditions of the region are classified as warm–humid, with an average temperature of 22.7 °C and an annual average precipitation of 2036.4 mm [36]. The region is situated at an elevation of 300 m above sea level.

2.2. Preparation of the Glyphosate Solution

To prepare the solution, 600 L of domestic wastewater was measured and mixed with glyphosate salts to achieve a concentration of 10 mg L^{-1} of glyphosate. The solution was placed in a 750 L tank, in which a transparent tube was installed to measure the water level and assess the hydraulic load. This system was used to supply water, dispense the solution, and maintain the required drip pressure. The experiment was conducted in two stages. In the first stage, the selected plants were adapted using only domestic wastewater collected from a sewer located within the campus. In the second stage, a mixture of domestic wastewater and glyphosate was used.

2.3. HSFW Design

The experimental system of a horizontal subsurface flow wetland (HSFW) consisted of a total of ten polypropylene cells (0.68 m long × 0.42 m wide × 0.35 m deep). These cells were arranged in two lines, with each cell in the second line serving as a replicate of the corresponding cell in the first line. The cells were assigned as follows: monoculture of *Alpinia purpurata*, monoculture of *Heliconia psittacorum*, monoculture of *Canna indica*, polyculture of the three ornamental species, and finally, a control cell in which no plants were planted (Figure 1). This design allowed for the evaluation of the performance of each treatment under replicated conditions. The control cell, without plants but with the same substrate, was maintained under the same operational conditions as the other cells, as all received the same supply from the feeding tank. This allowed for the evaluation of the impact of vegetation on contaminant removal.

Tezontle, or red gravel, was employed as the filtering material. The cell filling consisted of two layers. The first layer, composed of red gravel with a particle diameter ranging from 1.2 to 3.0 cm [37,38], had an average diameter of 10 cm and a porosity index of 0.66. The second layer consisted of 50 cm of red gravel, with a particle diameter between 0.8 and 1.0 cm and a porosity index of 0.62. The hydraulic retention time (HRT) was 3 days [39] (Figure 2).

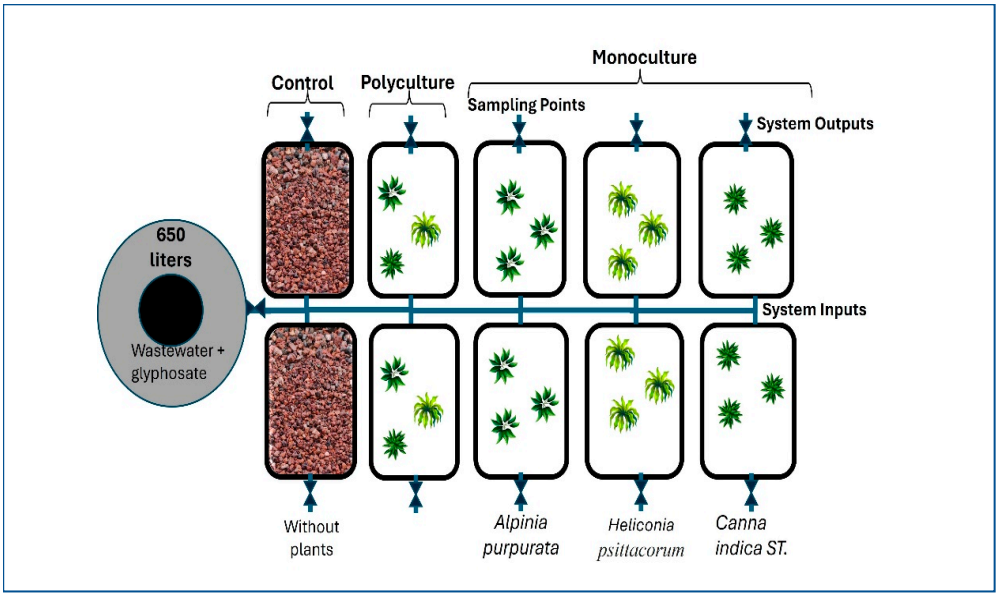


Figure 1. Visual representation of the design of mesocosms for the treatment of agricultural runoff contaminated with glyphosate.



Figure 2. Experimental system of constructed wetland mesocosms in open environment for the treatment of wastewater with glyphosate.

2.4. Plant Selection

Three plant species were used in the experiment, obtained through different methods. *Heliconia psittacorum* (31.6 ± 5.7 cm average height) was collected in its natural state from an area near the experimental site [36]; *Canna indica* (64.5 ± 3.5 cm average height) and *Alpinia purpurata* [40,41] were selected for their resistance to dissolved substances in wastewater and their role as hydrophytic plants, including their rhizomatous development, transport mechanisms, and nutrient transformation capabilities.

These species are commonly found in regional water bodies, where they thrive in direct sunlight at temperatures ranging from 25 to 32 °C and a relative humidity above 80%. Their primary nutrients include nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), and sulfur (S). They are capable of withstanding high chemical oxygen demand (COD) loads, as well as heavy metals, arsenic, and some reported emerging pollutants [42]. The three species are also valued for their aesthetic appeal and are used as ornamental plants, making them of significant commercial value (Figure 3).



(**a**) (**b**)

Figure 3. (**a**) Necrotically damaged leaf and (**b**) Alpinia pupurata developed to a lesser extent during the glyphosate wastewater treatment experiment.

2.5. Parameters and Measurements of Pollutants

2.5.1. Plant Development in Mesocosms

The development of the selected plants was analyzed through the following parameters: (a) the number of plants in each bush, (b) stem thickness, (c) height, (d) number of leaves, (e) leaf length, (f) leaf width, and (g) number of flowers, over a period of 5 months. Additionally, the health of the plants was assessed through visual inspection, focusing on their color and the presence of pests, such as ants, butterflies, caterpillars, spiders, and others.

2.5.2. Biomass in Mesocosms

For this analysis, each plant was extracted from the cells, and the roots were washed and separated from the aerial parts. The material was then weighed using a balance (OHAUS Compass, H-8111, Uline, Miami, FL, USA). After weighing, the samples were placed in paper bags, which were properly labeled for identification. Subsequently, the bags were placed in a dryer and maintained at 80 °C ± 2 °C for 1 day until they reached a constant weight. The reported value was obtained using the equation described below to determine the trend in each planting system. This process was carried out at the Water, Soil, and Plant Laboratory of the Instituto Tecnológico de Úrsulo Galván.

Plant biomass was calculated from the following equation [43] (Sandoval et al., 2021).

$$TB = (NP \times (AB + RB))/0.285$$

where:

TB = Total biomass, expressed in grams of dry weight per unit.
AB = Average biomass of the aerial part (expressed as bpd/plant).
RB = Average biomass of the root part (expressed as bpd/plant).
NP = Number of plants counted in the experimental unit (expressed as plant).
0.285 = Surface area of each cell (expressed in m^2).

2.6. Measurement of Physicochemical Parameters in Water

2.6.1. Physicochemical Parameters

The physicochemical parameters of the water, including the hydrogen potential (pH), temperature (°C), electrical conductivity (EC, µS cm^{-1}), and total dissolved solids (TDS, mg L^{-1}), were measured in situ using a HANNA multiparameter device version 11 (model HI 9828, USA). Samples were collected in duplicate from the tank and each of the system's cells. The wastewater quality parameters evaluated are shown in Table 1. The samples

were processed immediately after sampling in the Constructed Wetlands and Sustainability Laboratory of the TecNM, Misantla Campus.

Table 1. Contaminants analyzed and methods employed for their quantification and evaluation in this study.

Parameter	Method	Reference
pH	Electrode	[44]
Temperature (°C)	Thermometer	[45]
EC (µs cm^{-1})	Electrode	[46]
COD (mg L^{-1})	Closed Reflux	[47]
NO_2	Spectrophotometric	[48]
NO_3	Cadmium Reduction technique	[49]
NH_4	Nessler	Adapted from [50]
TN (mg L^{-1})	Kjeldahl Digestion Method	[51,52]
TP (mg L^{-1})	Ascorbic Acid Method	[53]

2.6.2. Glyphosate Analysis

Samples were collected every 20 days (a total of 8 samples throughout the study). A 0.5 L sample was taken from the feeding tank and one from each mesocosm. Although samples were collected every 20 days, the hydraulic retention time (HRT) of 3 days was respected at all times. That is, each outlet sample corresponded to the water that had entered the system three days prior, ensuring that the sampling accurately reflected the system's behavior within the established HRT. Water samples were collected in amber glass bottles that had been previously cleaned with diluted HCl (1%), and were then stored at 4 °C until analysis.

The water samples were centrifuged at 10,120 g (RFC) for 5 min to remove suspended particles. Subsequently, 10 mL of KH_2PO_4 (0.1 M) was added to each sample. The mixture was then filtered through a cellulose membrane filter (8 µm pore size, Whatman®, Guadalajara, México), which was washed three times, with the washes being combined. The pretreated water samples were then diluted with HPLC-grade water. Samples not treated with GLY were diluted 1:10, while treated samples were diluted 1:100,000. GLY was quantified using a commercial ELISA kit [54] (Glyphosate ELISA, Microtiter plate, Abraxis Inc., Leipzig, Alemania), following the manufacturer's instructions. The calibration curve was fitted using nonlinear regression to the four-parameter logistic equation (4-PL) with SoftMax Pro 7.1 software (Molecular Devices LLC, 2016).

The percentage of GLY present in each matrix was calculated relative to the initial GLY concentration in the tank (10 mg L^{-1}, representing 100%) and its detection in the outlet water (or fine gravel). The removal percentage (Rp %) was calculated using data from the outlet water [55].

$$Rp\ \% = \left(\frac{Concentration_{initial\ tank} - Concentration_{outlet\ water}}{Concentration_{\ initial\ tank}}\right) \times 100$$

2.7. Experimental Design and Statistical Analysis

The data were analyzed using a two-way ANOVA with Minitab® v.16 (Minitab Inc., State College, PA, USA). Prior to performing the analysis, the fundamental statistical assumptions were verified. The normality of the data was assessed using the Shapiro–Wilk test, and the homogeneity of variances was checked with Levene's test. It was assumed that both assumptions were met to proceed with the ANOVA analysis.

Subsequently, Tukey's pairwise comparison test was applied at a significance level of $p \leq 0.05$. Additionally, the effect sizes (η^2) and 95% confidence intervals for the means of each treatment were calculated, providing a more comprehensive and robust context for the results. A completely randomized design was used, where each experimental cell, along with its corresponding replica in the second line of mesocosms, was considered as an independent experimental unit. This design allowed for the evaluation of the treatment effects under replicated conditions.

3. Results

3.1. Plant Development

3.1.1. Plant Growth and Biomass Production

The main visible effects observed in the studied species included reduced growth, leaf loss, chlorosis, and, in some cases, the necrosis of plant tissues. These symptoms indicated a deterioration in plant health, likely related to the presence of glyphosate. Chlorosis, characterized by the yellowing of leaves due to chlorophyll deficiency, and necrosis, which results in the death of plant cells, were indicative of significant damage to the fundamental physiological processes of the affected plants.

In Figure 3a, necrosis is observed at the tip and near the midrib of the leaf. However, the plant managed to adapt and remained viable throughout the study, despite experiencing leaf loss. In Figure 3b, the smaller size of *Alpinia purpurata* is evident in comparison to *Canna indica*, which, over a 40-day period, tripled the height of *Alpinia purpurata*.

Plants in constructed wetlands play an essential role in the uptake and removal of waterborne pollutants, including nutrients, heavy metals, and organic compounds. Through processes such as phytoextraction and phytodegradation, plants are capable of sequestering and transforming these contaminants, thereby enhancing the overall quality of the treated effluent. The incorporation of ornamental plant species in constructed wetlands not only contributes to aesthetic value, but also influences growth patterns and reproductive output, which are key factors in evaluating system performance. Table 2 presents a comprehensive overview of plant development throughout the duration of the experiment.

Table 2. Growth of *C. indica*, *H. psittacorum*, and *A. purpurata* in the period of analysis in mesocosm systems with monoculture and polyculture.

Variable	Monoculture Mesocosm						Polyculture Mesocosm					
							C. indica + H. latispatha + A. purpurata					
	C. indica		*H. latispatha*		*A. purpurata*		*C. indica*		*H. latispatha*		*A. purpurata*	
	i	*f*	*i*	*f*	*i*	*f*	*i*	*f*	*i*	*f*	*i*	*f*
Number of Leaves	25	65 [a]	15	39 [a]	125	308 [a b]	18	58 [a]	8	21 [a]	99	230 [a]
Number of flowers	0	18 [a]	0	0	0	6 [a b]	0	24 [a]	0	0	0	0

C. indica: Canna indica; *Heliconia psittacorum*: H. psittacorum; *Alpinia purpurata*: A. Purpurata;i:initial; *i:* initial, *f*: final: [a] significant difference between *i* and *f*. [b] Significant difference between treatment.

In a study conducted by [56], a significant difference in yield was observed depending on whether the plants were grown in monoculture or in combination with other species. He highlighted the competitive dynamics in mixed cropping systems, noting that *Canna indica* outperformed *Schoenoplectus validus* as the superior competitor. The same trend was observed in this study (Table 2). *Canna indica* exhibited the most significant flower development (Figure 4), both in monoculture and polyculture. This behavior may be

attributed to its competitive advantage in the polyculture system, as discussed by the author.



Figure 4. From the beginning of the experiment, Canna indica showed adaptation to the conditions, maintained its growth of stem, leaves and flowering.

At the end of its cycle, Canna indica reached a height and flowering similar to those it exhibits naturally in the region. According to the records, significant biomass was generated, demonstrating its ability to withstand and adapt to the conditions of wastewater and glyphosate.

In this study, *Heliconia psittacorum* did not produce flowers (Figure 5), which could be attributed to the novel conditions it was exposed to, including the complex nutritional conditions and the characteristics of the water provided, as well as the space in which it was planted.

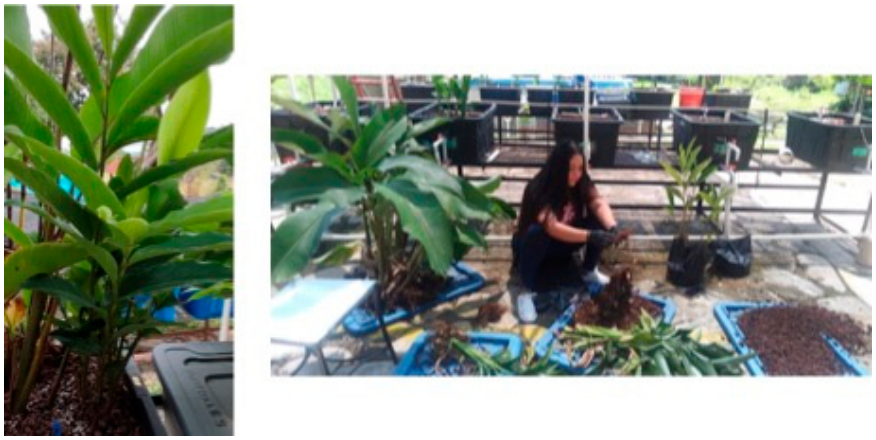


Figure 5. *Heliconia psittacorum*., with abundant biomass, number of leaves, and number of plants in each bush. It did not reach a flowering cycle.

Alpinia purpurata, in both systems, demonstrated the most substantial foliage development; however, this species is known for its large leaf production (Figure 6). *Alpinia purpurata* only developed flowers in monoculture, but the number of flowers was greater than those reported by [40]. Similarly, in the study by [37], no flowers were produced. They observed that the plants did not exhibit the same growth behavior as in their natural habitat, which may have been due to the altered conditions under which they were cultivated, both in monoculture and polyculture. Consequently, further studies with extended evaluation periods are necessary to determine the flowering rate of these species when used in constructed wetlands for ornamental purposes.



Figure 6. Development of Alpinia Purpurata, presenting abundant biomass and flowering that evidences its maturity cycle during the experiment.

None of the plants were replaced, as all of them successfully adapted to the initial phase and subsequently to the second phase, which involved the addition of GLY. Additionally, no pest infestations were observed throughout the study.

The *Canna indica*, *Heliconia psittacorum*, and *Alpinia purpurata* plants used in the experimental units exhibited robust growth within the system. Although *Canna indica* plants grew significantly taller, the growth rates of *Heliconia psittacorum* and *Alpinia purpurata* did not differ statistically ($p < 0.05$) (Figure 7). These findings align with the study by [57], where *Canna generalis*, a plant from the same genus, demonstrated superior growth compared to *Heliconia psittacorum*. The increase in plant productivity can primarily be attributed to the greater availability of water, light, and nutrients [58].

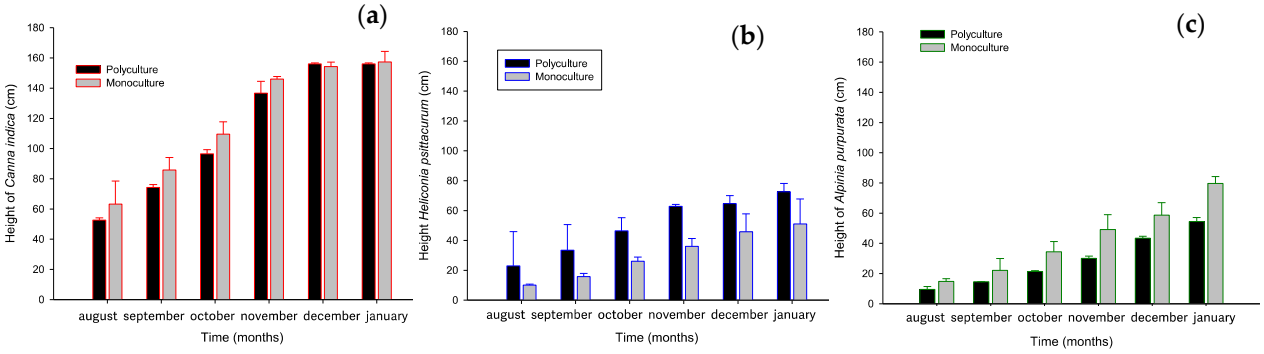


Figure 7. Growth of (**a**) *Canna indica*, (**b**) *Heliconia psittacurum*, and (**c**) *Alpinia purpurata* planted in the systems during the study.

Canna indica exhibited vigorous development in both monoculture and polyculture systems, while *Heliconia psittacorum* thrived more in polyculture and *Alpinia purpurata* showed better growth in monoculture. This difference in performance may have been due to the species' varying sensitivities to environmental changes, with *Heliconia psittacorum* and *Alpinia purpurata* demonstrating slower adaptation compared to *Canna indica*. It is recommended that a long-term study be conducted to further investigate the growth rates of these species under different cultivation systems.

3.1.2. Biomass

The species used in the study were harvested, and both the wet and dry weights of the aerial and below-ground biomass were measured. Wetland biomass holds significant potential for various applications, including resource recovery, environmental restoration, and as a sustainable feedstock for energy production. In this study, biomass was evaluated to determine whether the presence of GLY could inhibit plant development or even induce

species mortality. In this context, the plants adapted without significant issues. Table 3 presents the wet and dry weights of the plants at the conclusion of the study.

Table 3. Biomass development of *Canna indica*, *Heliconia psittacorum*, and *Alpinia Purpurata* in monoculture and polyculture.

Cultivation Method	Plant Specie	Fresh Biomass (g)	Dry Biomass (g)
Monoculture	*Canna indica*	8215.1	292.8
	Heliconia psittacorum	3115.0	786.0
	Alpinia Purpurata	7320.8	3053.0
Polyculture	*Canna indica*	4960.5	481.0
	Heliconia psittacorum	7395.9	1723.0
	Alpinia Purpurata	1685.8	498.0

Wetland biomass is known to exhibit a significant concentration of nitrogen, a key nutrient that plays a crucial role in various ecological processes such as primary productivity, plant growth, and nutrient cycling [59]. The high nitrogen content in wetland plants is often attributed to the plants ability to efficiently uptake and assimilate nitrogen from the surrounding environment, particularly from water and sediments enriched with nitrogen compounds. As shown in Table 3, *Canna indica* and *Alpinia purpurata* developed a greater biomass in monoculture compared to polyculture, while *Heliconia psittacorum* displayed the opposite trend. Throughout the study, *Canna indica* demonstrated stable growth, with the production of numerous new shoots (Table 3). *Canna indica* exhibited a higher fresh biomass in monoculture, but a lower dry biomass, in contrast to *Heliconia psittacorum*, which demonstrated the opposite trend. This discrepancy suggests that, while *Canna indica* may have had a greater initial growth in terms of water content, its biomass accumulation in terms of dry weight was less pronounced compared to *Heliconia psittacorum*. Similarly to the study by [60], where *Heliconia* exhibited a faster growth and greater height than *Canna hybrids*, in this study, *Heliconia psittacorum* showed a higher dry biomass than *Canna indica*. These developmental differences may be attributed to the physiological advantages of *Heliconia* spp., such as a greater efficiency in nutrient uptake and utilization, a faster growth rate, and better adaptation to stress conditions. This reinforces its potential as an ornamental species for the phytoremediation of highly contaminated effluents in constructed wetlands [61].

3.2. Measurement of Physicochemical Parameters in the Water

The average pH of the water from inlet A was 7.40 $\pm$ 0.20. After passing through the treatment in the control system, the average pH increased to 7.94 $\pm$ 0.09, indicating a tendency towards alkalinity. This increase could be attributed to the type of substrate, the hydraulic retention time, and the presence of GLY. On average, the pH of all systems was 7.42 $\pm$ 0.20, with no significant differences between treatments (monoculture and polyculture). At lower pH levels, the concentrations of ammonia and nitrate in the effluent tended to increase, which may indicate a disruption in nitrification and denitrification processes. However, in this study, pH did not appear to influence these processes, as nitrogen compounds significantly decreased in the evaluated treatments.

The average conductivity values throughout the study at the inlet were 1341.7 $\pm$ 10.02 $\mu S\ cm^{-1}$. After treatment in the various systems, the following results were observed: the control system had a conductivity of 1207.10 $\pm$ 25.15 $\mu S\ cm^{-1}$, the polyculture had 933.6 $\pm$ 42.08 $\mu S\ cm^{-1}$, the *Alpinia purpurata* monoculture had 984.4 $\pm$ 66.50 $\mu S\ cm^{-1}$, the *Canna indica* monoculture had 1038.8 $\pm$ 54.50 $\mu S\ cm^{-1}$, and the *Heliconia psittacorum* monoculture had 1100.4 $\pm$ 87.43 $\mu S\ cm^{-1}$. Statistical analysis revealed no significant differences between the inlet and outlet for the *Heliconia* and *Alpinia purpurata* monocultures, but

significant differences were observed between the inlet and outlet for Canna indica. The most notable difference was found between the inlet and outlet of the polyculture, which showed the superior treatment of wastewater with GLY. The variation in electrical conductivity (EC) in constructed wetlands can be attributed to various physicochemical processes, such as ion removal through sedimentation, precipitation, adsorption, and absorption by plants. The decrease in conductivity after treatment could be associated with a reduction in the total dissolved solids (TDSs) concentration and the conversion of NO_3-N into molecular nitrogen (N_2), leading to a decrease in charged ions [62].

In some studies, the reduction in TDSs after treatment is typically attributed to solids deposition caused by retention time, as well as the absorption of dissolved solids by wetland plants. However, in this study, an increase in TDSs was observed, which could have been due to the presence of organic matter, the decomposition of plant remains, and ion deposition within the wetland [63]. The results indicated that the highest TDS removal efficiency was observed in the polyculture, with a 23% removal rate. A significant difference was found between the inlet (804.02 $\pm$ 25.25 ppm) and the polyculture (613.14 $\pm$ 17.25 ppm), as well as between the inlet and the monocultures of *Canna indica* (624.73 $\pm$ 27.64 ppm), Alpinia purpurata (671.72 $\pm$ 32.90 ppm), and *Heliconia psittacorum* (630.16 $\pm$ 42.37 ppm), although no significant differences were found between the treatments. The removal percentages did not exceed 30%.

The chemical oxygen demand (COD) showed a significant difference between the treatments and the control, indicating that plant-based treatments were more efficient. There was no significant difference in COD reduction between the plant species used, suggesting that the choice of plant species did not significantly affect the performance of the system. Similarly, no significant differences were observed between the monoculture and polyculture systems for this parameter. Regardless of the chosen configuration, the results were similar. The mean COD concentration at the system inlet was 567 $\pm$ 65 mg L^{-1}, while at the outlet, it was 141 $\pm$ 29 mg L^{-1}. These results are comparable to those reported by [64] (inlet concentration of 330 mg L^{-1} and outlet concentrations of 81.8 mg L^{-1} and 89.8 mg L^{-1}), showing a similar removal percentage, particularly when using Canna indica.

There are limited studies currently employing *Heliconia psittacorum* in constructed wetlands; however, this study recorded a 77% removal rate for chemical oxygen demand (COD), which is consistent with other studies reporting similar results, such as 78% COD removal [65]. In a study by [57], COD removal ranged from 75.5% $\pm$ 7.9% for *Canna indica* to 75.3% $\pm$ 9.0% for *Heliconia psittacorum*.

The system with the lowest efficiency for ammonium (NH_4^+), nitrate nitrogen (N-NO_3), and nitrite nitrogen (N-NO_2) removal was the control. Conversely, the system planted with *Canna indica* demonstrated the highest efficiency in ammonium removal. However, no significant differences were observed among the various systems, except between the control and the plant-based systems. For N-NO_3, the system with the lowest efficiency was the control, showing a significant difference compared to both the plant and non-plant systems. Furthermore, the *Heliconia psittacorum* system exhibited significant differences in efficiency compared to other treatments. As for N-NO_2, the control system again showed the lowest removal efficiency. The systems planted with *Heliconia psittacorum* and *Alpinia purpurata* exhibited the highest removal efficiencies, with statistically significant differences compared to the other treatments. It is important to note that the roots of *Canna indica* possess a high sorption capacity for nitrogenous nutrients [64], which likely contributes to its higher efficiency as a wetland plant.

In a study by [66], *Alpinia purpurata* was shown to remove 17.2 $\pm$ 1.3% of N-NO_3, which represents an efficiency four times lower than that observed in the current study. This suggests a significant improvement in the efficiency of *Alpinia purpurata* in our research.

A subsequent study by the same authors [67], where *Alpinia purpurata* was used in both monoculture and polyculture, did not provide specific data on N-NO_3 removal. In some cases, nitrate concentrations tend to increase. For instance, ref. [68] observed that, initially, nitrate concentrations were 3.34 mg L^{-1}, but after 144 h of treatment with *Canna indica*-planted wetlands, the concentration increased to 8.64 mg L^{-1}. This increase in nitrate concentration could indicate ongoing nitrification processes in the treated wastewater.

The control system, which contained only the substrate, exhibited a lower pollutant removal efficiency compared to treatments that incorporated plants. Statistically, any of the plant-based treatments can be effectively utilized for total nitrogen (TN) removal, yielding excellent results. In this study, the TN removal percentages exceeded those reported by [28], which achieved only 60.40 ± 5.60%. This indicates that all three plant species used in this research possess a high TN removal capacity, both in polyculture and monoculture, making them viable candidates for wastewater treatment.

Canna indica, in particular, has demonstrated the ability to remove up to 97% of TN in vertical flow-constructed wetlands [69]. The authors highlight that *Canna indica* is highly suitable for wastewater treatment in tropical regions due to its efficiency and adaptability. Additionally, *Canna indica* is an ornamental species, making it an aesthetically appealing option for constructed wetlands. Ref. [57] evaluated the efficiency of a vertical flow-constructed wetland (VFCW) system for municipal wastewater treatment, using either *Canna indica* or *Heliconia psittacorum*. Their results showed TN, NH_4-N, and total phosphorus (TP) removal efficiencies exceeding 40% when using *Canna indica* (44.3 ± 5.3%, 56.9 ± 13.4%, and 56.7 ± 8.2%, respectively) and *Heliconia psittacorum* (38.7 ± 2.7%, 50.0 ± 9.4%, and 49.1 ± 7.3%, respectively). However, these values were lower than those obtained in the present study (Table 4).

Table 4. Efficiency of contaminant removal in wastewater using monoculture and polyculture treatments of tropical plants.

Treatments	COD	Ammonium	N-NO_3	N-NO_2	TN	TP	PO_4^{-3}
Inlet	567 ± 65	51 ± 15	27 ± 2	45 ± 3	138 ± 26	28 ± 2	14 ± 1
Control	377 ± 80	26 ± 6	16 ± 2	38 ± 6	77 ± 9	16 ± 1	10 ± 2
% efficiency	41	49	41	16	44	43	29
Policulture	139 ± 28	3 ± 1	6 ± 1	7 ± 2	16 ± 4	3 ± 0	1 ± 0.6
% efficiency	75	94	78	84	88	89	93
Alpinia Purpurata	128 ± 18	5 ± 3	7 ± 2	4 ± 3	18 ± 5	3 ± 1	0.31 ± 0.15
% efficiency	77	90	74	91	87	89	98
Heliconia psittacorum	130 ± 12	6 ± 2	5 ± 1	4 ± 1	17 ± 3	3 ± 1	1.2 ± 0.2
% efficiency	77	88	81	91	88	89	91
Canna indica	141 ± 29	3 ± 1	6 ± 2	5 ± 1	13 ± 4	2 ± 0	0.34 ± 0.09
% efficiency	75	94	78	89	91	93	98

The control system exhibited the lowest removal efficiencies for TP and phosphate (PO_4^{3-}), underscoring the critical role of plants in pollutant removal within constructed wetlands. Statistically, all treatments demonstrated excellent results, making them viable options for PO_4^{3-} removal. While *Canna indica* achieved the highest TP removal, the systems planted with *Alpinia purpurata* and *Canna indica* were the most effective in PO_4^{3-} removal, showing significant differences compared to the control, polyculture, and *Heliconia psittacorum* system. *Alpinia purpurata* and *Canna indica* achieved the highest removal percentages (98%) (Table 4).

Alpinia purpurata exhibited a significant difference (28.8% ± 36%) in PO_4^{3-} removal compared to previous studies by [67], which reported a removal efficiency of 78.2% ± 10.9%. The concentration of PO_4^{3-} decreased from 13.17 mg L^{-1} at day 0 to 0.20 mg L^{-1} at day 200 of experimentation. Furthermore, the polyculture system also demonstrated a high PO_4^{3-} removal efficiency, surpassing the results obtained in the aforementioned studies. The 98% removal efficiency observed aligns with the findings of [69], whose reported values were slightly higher than those obtained in this study (Table 4).

Based on these findings, *Canna indica* emerges as the most promising candidate for the efficient removal of phosphorus pollutants in constructed wetlands.

3.3. Glyphosate Removal

In Mexico, pesticide use is a widespread practice, with GLY being one of the most commonly used herbicides. However, on 31 December 2020, the Mexican government issued a decree banning the use of GLY, set to take effect in January 2024 [70].

Compared to previous studies that used iron- and calcium-enriched substrates, our results in control cells with red volcanic gravel showed the progressive removal of glyphosate, reaching 13.77% at 40 days and 49% at 200 days. In the study by [28], a pilot-scale constructed wetland system with an iron-rich substrate (Pyrr-CW) achieved a removal efficiency of 90.3%, while the system with a calcium-rich substrate (Lime-CW) reached 66.4%, with statistically significant differences between them. Due to these high removal rates, the authors identified substrate adsorption as the primary mechanism for glyphosate removal in constructed wetlands.

In our study, the removal efficiency in the control cells was considerably lower compared to these optimized systems, suggesting that red volcanic gravel has a limited adsorption capacity or that glyphosate degradation mechanisms, such as microbial activity, are restricted in the absence of plants. In fact, glyphosate removal was significantly lower in the control cells compared to in the vegetated cells, with statistically significant differences between both groups. These results highlight the key role of plants in contaminant removal and emphasize the importance of evaluating substrate type as a determining factor for glyphosate removal efficiency in constructed wetlands.

At the end of the 200-day experiment, a GLY concentration of 16.52 ± 0.5 ppm was recorded at the inlet. This increase in concentration can be explained by the gradual accumulation of the contaminant at the bottom of the wastewater container. However, the removal capacity of the plants was determined from this concentration. To ensure optimal pollutant homogenization in the water, maintaining a constant system agitation is essential.

Figure 8 clearly illustrates that, at the conclusion of the experiment, the highest removal efficiencies were recorded across all systems. In the polyculture treatment, removal rates of 57.11%, 71.03%, 51.11%, 64.13%, and 70.31% were observed at 40, 80, 120, 160, and 200 days, respectively. A significant difference was observed between the beginning and end of the experiment. Based on these findings, a polyculture system comprising *Canna indica*, *Alpinia purpurata*, and *Heliconia psittacorum* is not recommended for GLY removal from wastewater, as it did not demonstrate a superior performance compared to a monoculture system with *Heliconia psittacorum*.

At 120 days, *Heliconia psittacorum* demonstrated a greater stability and adaptation, resulting in a significant increase in its contaminant removal capacity from that point onward. By the end of the experiment, at 200 days, this species achieved the highest removal efficiency, reaching 97.10% (Table 5). This result exceeded those obtained by [71] who reported glyphosate removal efficiencies of 80%, 69%, and 59% in surface flow wetlands, suggesting that, in some systems, glyphosate removal rates may decrease over time. However, in our study, all systems showed a continuous improvement in removal efficiency throughout

the period. Additionally, our results were superior to those of [17], who used subsurface flow wetlands planted with *Phragmites australis* subsp. *americanus*, *Scirpus cyperinus*, and *Sporobolus michauxianus*, achieving glyphosate removal efficiencies ranging from 54% to 76% in systems with macrophytes and from 78% to 82% in systems with biochar. In this study, synthetic agricultural runoff containing 50 μg L^{-1} of glyphosate was applied. The difference in the removal efficiencies between the two studies can be attributed, in part, to the selected plant species and exposure time. In our study, the progressive adaptation and greater stability of *Heliconia psittacorum* over time likely contributed to its superior performance, whereas the biochar utilized in the study of [17] enhanced the glyphosate adsorption capacity, but did not achieve the same level of efficacy as the gradual adaptation observed in the plant within our system.

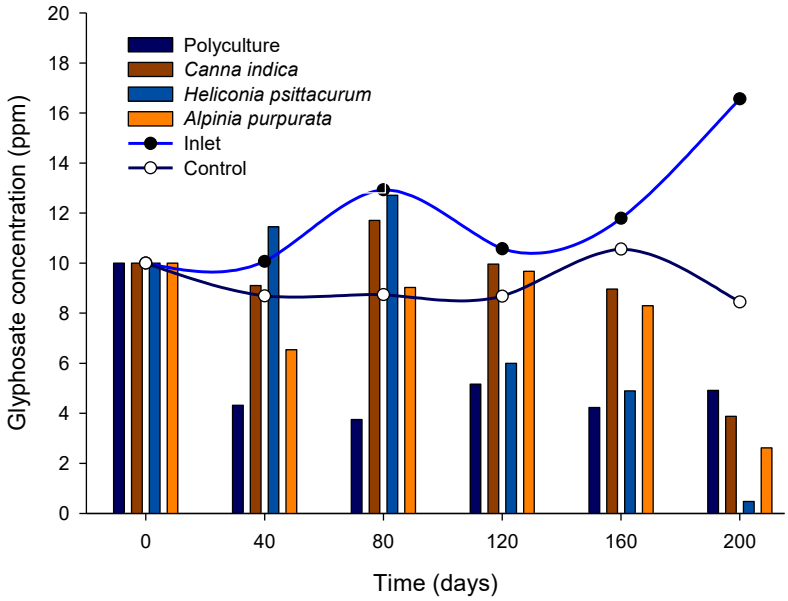


Figure 8. Timeline of GLY removal by *Canna indica*, *Heliconia psittacorum*, and *Alpinia purpurata* in polyculture from 0 to 200 days.

Table 5. Glyphosate removal efficiency in wastewater using monoculture and polyculture treatments of tropical plants.

Treatments	% Removal Efficiency				
	40 Days	80 Days	120 Days	160 Days	200 Days
Inlet (ppm)	10.07 ± 0.2	12.93 ± 0.5	10.57 ± 0.5	11.79 ± 0.3	16.56 ± 0.5
Control	13.77	32.42	17.92	10.46	49.00
Policulture	57.11	71.03	51.11	64.13	70.31
A. purpurata	35.05	30.21	8.54	29.58	84.19
H. psittacorum	No removal	1.75	43.25	58.44	97.10
Canna indica	9.65	9.44	10.51	23.98	76.60

The type of wetland and vegetation used can significantly influence pollutant removal. In this study, *Alpinia purpurata* exhibited the second-highest removal efficiency after 80 days of exposure. From that point onward, the GLY concentrations in the system gradually declined, ultimately reaching 84.19% removal. Previous studies have shown that subsurface flow wetlands can achieve removal efficiencies exceeding 90% and that incorporating hybrid flow components can further enhance the removal of agrochemicals such as GLY [71]. In our study, the removal efficiency improved as the experiment progressed.

Conversely, the *Canna indica* monoculture exhibited the lowest performance, with a final GLY removal efficiency of only 76.60%. Although removal efficiency peaked at the end of the experiment, significant differences were observed between this treatment and the others. The results suggest that the plants experienced an adaptation period during the study. After 80 days, the GLY concentrations in the outlet water of the treatment system exhibited a gradual downward trend, as depicted in Figure 5. This progressive decline in GLY concentration indicates that both the plants and the system as a whole were optimizing their ability to remove this pollutant from wastewater. Understanding these dynamics is essential for continuously improving the performance of constructed wetlands in removing contaminants such as GLY.

Several studies have evaluated the efficiency of constructed wetlands in removing GLY under different experimental conditions. Ref. [72] analyzed five GLY concentrations (0, 10, 25, 50, and 100 mg L^{-1}) and found that removal efficiency significantly decreased as the GLY concentration increased. However, vegetated devices exhibited a higher removal efficiency compared to non-vegetated devices when GLY concentrations were below 50 mg L^{-1}.

Ref. [73] highlighted that the improvement in removal efficiency was attributed to the increase in retention time. The removal of GLY exceeded 93%. Similarly, ref. [54] identified that *Panicum maximum* treated with glyphosate exhibited the highest removal efficiency, reaching 87%. These findings underscore the potential of constructed wetlands and plant species for glyphosate and other pesticide removal, emphasizing the importance of proper system design to maximize their efficiency in the bioremediation of contaminated water. Concentrations higher than those obtained in this study (Table 5) were observed in some cases.

4. Conclusions

The plants demonstrated a GLY removal capacity with an efficiency exceeding 50% (based on experimental data) through root absorption. The results indicate that the studied plant species reduced the initial concentration of this contaminant by 70% over a 200-day period, highlighting their potential for the phytoremediation of agricultural runoff. Although all plant species contributed to GLY removal, *Heliconia psittacorum* exhibited the highest removal rate, whereas the same species in polyculture showed a lower efficiency.

No visible changes in plant health or overall appearance were observed during GLY applications, even at concentrations of up to 8 ppm after 200 days of exposure, suggesting a high tolerance to this herbicide.

The scalability of this system is promising, as the species studied, such as *Heliconia psittacorum* and *Alpinia purpurata*, demonstrated the efficient removal of glyphosate and other pollutants under mesocosm-scale conditions. The high removal efficiencies of nutrients and glyphosate, along with the plants' ability to thrive in both monoculture and polyculture, suggest that this system could be adapted to larger scales, such as constructed wetlands in the field. However, the effectiveness of large-scale removal will depend on factors such as plant density, wetland design, and environmental conditions, which should be evaluated through further studies in more complex, long-term scenarios.

Further studies are recommended with extended experimental periods exceeding six months and GLY concentrations of 5, 10, 20, 40, and 50 ppm to assess long-term removal capacity. Additionally, evaluating at least three additional plant species, preferably native and with ornamental value, would be beneficial to explore their efficiency in GLY removal and their applicability in phytoremediation projects.

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Writing—original draft, G.A.-C. and L.C.S.H.; Writing—review & editing, G.M.-R. and L.C.S.H.; Visualization, M.C.M.-P.; Supervision, G.A.-C.; Project administration, G.M.-C. All authors have read and agreed to the published version of the manuscript.

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