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# Evaluation of a wetland species: *Cyperus giganteus* vahl (Poales – Cyperaceae), propagation and efficiency in effluents bioremediation

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## ABSTRACT

The Ypacarai Lake is located in the City of San Bernardino, Cordillera Department, Paraguay. This lake is part of an important system of wetlands that covers a large area, and is of vital ecological importance due to its rich biodiversity and its numerous roles in the regulation of environmental adaptation and maintenance. However, in recent years this system has suffered the onslaught of pollution due to the discharge of highly contaminated effluents. It is known that this type of complex and fragile ecosystems in the face of degradation processes, such as drainage for agricultural use and civil works, which on various occasions cause alterations in its natural dynamics, such as a decrease in the capacity to regulate water quality, loss of endemic species, among countless other impacts on the environment. In this study, an endemic species of this wetland system, *Cyperus giganteus*, was studied, mainly addressing the best time to collect seeds for plant production, the viability of its seeds and the power of biopurification of household effluents, since it is the largest type of discharge that goes to this wetland system. In this research work, it was shown that the best time for fruit collection is the month of December, obtaining a percentage of seed germination of 72%, that in the accelerated aging test, the seed vigor is moderate, that the species *Cyperus giganteus* has optimal properties to reduce the pollution load of household effluents and it was demonstrated that it will be appropriate to repopulate some areas of the wetland with this species.

## INTRODUCTION

The RAMSAR convention defines wetlands as "Extensions of marshes, swamps and peat bogs, or surfaces covered with water, whether natural or artificial, permanent or temporary, stagnant or flowing, fresh, brackish or salt, including extensions of sea water whose depth at low tide does not exceed six meters"**(Ramos & Uribe 2009)**.

Wetland features can be grouped into components, functions and properties. **(Hernández *et al*., 2009)**. Wetlands provide effective nutrient sinks and buffer sites for organic and inorganic contaminant, andare considered natural media that filter or help improve water quality **(Barboza 2000; Frers 2008)**.

Due to their rich biodiversity and the numerous regulation and support functions that they develop, wetlands arecomplex and fragile ecosystems whenfacing degradation processes, such as drainage for agricultural use and civil works, which on several occasions cause alterations in their natural dynamics, such as a decrease in the ability to regulate water quality, loss of endemic speciesand accumulation of sediments. Wetlands provide ecosystem services such as habitat for a great diversity of species, water supply and the ability to regulate water quality **(RAMSAR, cited by Aranda *et al*., 2012)**.

Phytopurification is a wastewater purification system, based on the use of artificial wetlands in which aquatic plants (hydrophytes) are developed toactivelycontribute in theelimination of pollutants, mainly organic matter. These systems are not expensive, considering the initial investment and maintenance, and comprise a sustainable technology for cleaning up the environment. Its approach is based on a wide range of plants including terrestrial, aquatic, annual, perennial (agricultural crops and trees), ornamentals, and weeds **(Velázquez *et al*., 2010; Lichtfouse 2015)**.

The Cyperaceae family species are considered emergent plants that have their roots in the soil below the water column, while their stems, leaves (photosynthetic organs) and reproductive organs are aerial **(Martínez *et al*., 2012)**. The *Cyperus giganteus*Vahl (Poales, Cyperaceae) is an aquatic species with rhizomesthat develop in channels or shallow water holes, with permanent water and quickly propagate vegetatively or through seed dissemination **(Méreles 2006; Benítez *et al.*, 2009)**.

According to **Pin *et al*., (2009)**, *C. giganteus* is a native herbaceous plant, approximately 2 meters tall, with subterranean stems or horizontal rhizomes that fix it to the ground, and aerial stems very close together, that are serial, cylindrical, triangular in the upper part, smooth and covered at their lower extremity with red, leathery sheaths. They lack leaves, except for the two upper ones, which are sometimes rudimentary. Yellowish or greenish flowers or spikes protected by glumes, arranged in cylindrical spikes arranged, in turn, in umbels, surrounded by leaves-like bracts. Elongated achene fruits, with three faces, grayish, smooth and ending in points.

The aim of this research is to establish the best season for collecting seeds for reproduction, to evaluate the seeds viability and the phytoremediation capability of the species to be use in contaminated water systems recovery.

## MATERIALS AND METHODS

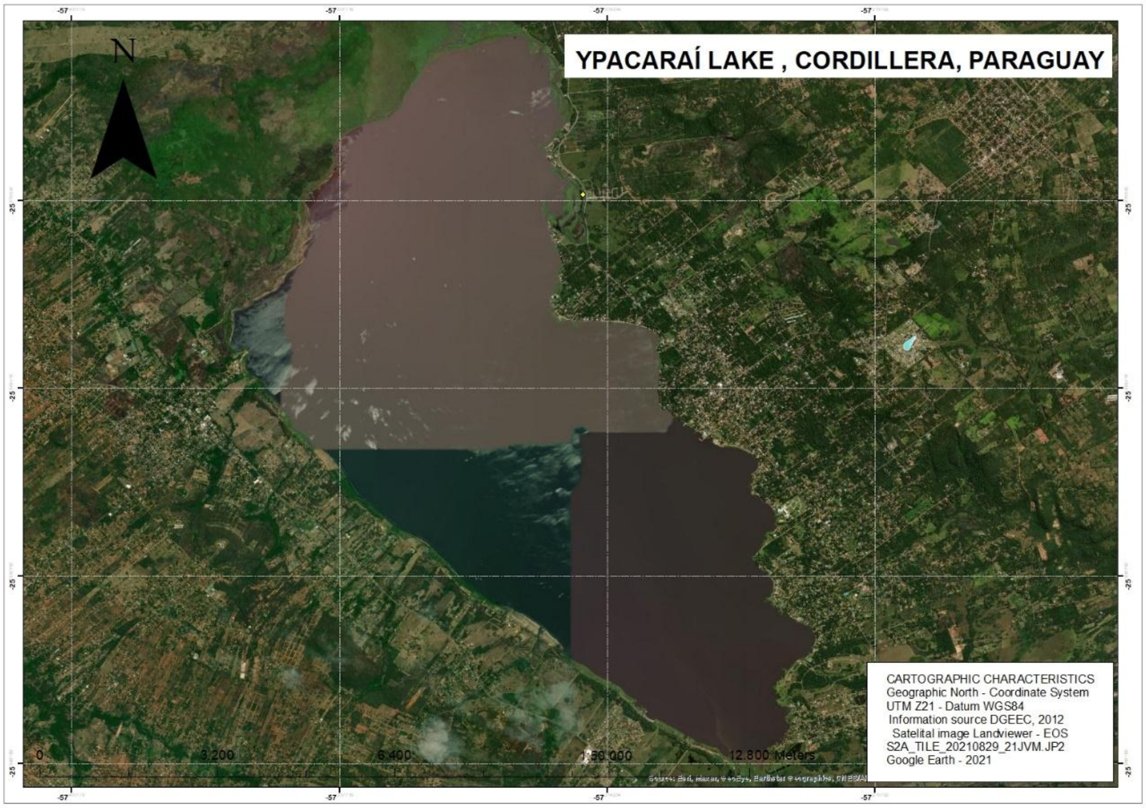


**Fig.1.** Satellite image of Ypacarai Lake, City of San Bernardino, Central Department, Paraguay.

Plants of *C. giganteus* located in coastal vegetation on the Ypacarai Lake, Cordillera, Paraguay, at coordinates 25°16'26.5"S 57°19'05.5"W were selected. The sampling point was chosen under accessibility criteria and taking as reference the frequency and abundance of the species, a population size of at least 1000 plants.

### Seed collection

Seed were collected between the years 2018/2019, five times, with 30-day intervals:

1- September 18

2- October 18

3- November 18

4- December 18

5- January 18

These dates were established according to the seeds maturation rangeof the species studied. The spikes were collected from the sampling point, and those with a good sanitary appearance were taken and the color they presented at the time of collection was recorded. The samples were placed in brown paper bags, labeled with location and date of collection. Later, they were transferred to coolers for processing. The spikes were cut and placed in Petri dishes with absorbent paper, in a number of 10 spikes from the same inflorescence, then were stored at room temperature (21°C) until sowing.

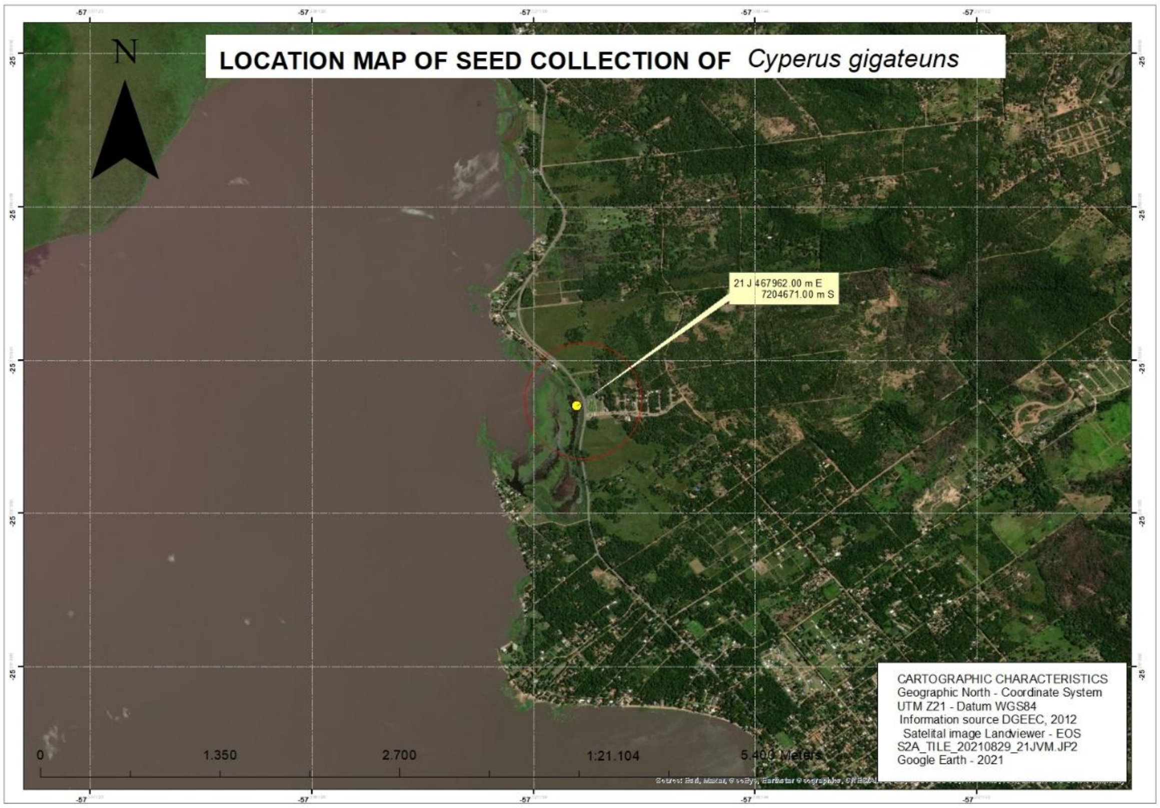


**Fig.2.** Location map of the *C. giganteus* seed sampling site on the river of the Ypacarai Lake, City of San Bernardino, Central Department, Paraguay.

### Propagation of *C. giganteus* in a vegetation house

For propagation by seeds, Ypararai Lake soil collected from the lake river was used as substrate, which was sterilized in an autoclave for 50 minutes, at 1 atm of pressure and at a temperature of 121°C.

For sowing the seeds, plastic trays with capacity of 55 cc were used, which were fully loaded with sterile substrate, then seeds were sown at a depth of 0.5 cm. The experimental unit consisted in a tray with 20 seeds, in 20 repetitions, giving a total of 400 seeds. Once the seeds were sown, the trays were placed in plastic containers with water in order to simulate its natural habitat conditions of development. This step was performed for each seed collection date, as mentioned above.

The transplant was carried out 35 days after sowing (DAS), each emerged seedling was transplanted into a 250 mL plastic cup and kept in plastic containers with water, cups were previously prepared with holes in the lower part so water could be absorbed.

**Greenhouse evaluations. Germination percentage, development and survival percentage**

Thirty days after sowing, germination percentage was evaluated, considering emerged seedlings that showed normal development. After transplantation, the percentage of plants that survived was evaluated. For establishment after transplantation, the percentage of plantsthat survived was evaluated as well as the average height at 60 days after transplantation.

**Seed vigor estimation by accelerated aging method**

The accelerated aging test was carried out according to the Manual of Vigor Tests **(International Seed Testing Association, 2014)**. The seeds were subjected to thermal stress, for the test was selected the lot that corresponded to the date of seed sampling with the highest germination percentage. The stress consisted in subjecting seeds to different time and temperatures intervals through a chamber: t1: seeds without stress; t2: seeds at 40 °C for 12 hours; t3: seeds at 50 °C for 12 hours; t4: seeds at 45 °C for 24 hours; t5: seeds at 55 °C for 24 hours. After treatment, all seeds were sown in gerboxes on moist paper towels and kept in a culture room at a constant temperature of 26 °C. At day 30 after sowing, the germination percentage of all treatments was determined.

**Phytoremediation capability**

To evaluate the plants pollutansabsorption efficiency, were used household effluents. Black water samples were taken from a settling chamber, which contained waste water from sink and kitchen. The trial consisted in evaluating different water quality parameters of the effluents with the *C. giganteus* plants in three treatments: T1: control, 18 liters of drinking water without effluent; T2: 18 liters of solution of 50% effluent + 50% drinking water; T3: 18 liters of 100% effluent. The experimental unit consisted in a 20 liters container capacity with three C. giganteus adult plants, with the treatments described in three repetitions. Measurements were made on days 1, 7 and 14, considering the physicochemical and microbiological parameters (BOD, COD, NTK, PT, pH, temperature, conductivity, dissolved oxygen and fecal coliforms), these parameters were considered in relation to the standards established for final disposal of water.

### Data analysis and interpretation method

For data interpretation, the Analysis of Variance (ANAVA) was aplied to determine the differences between the treatments effects. Also a means comparison was made by Tuckey test to obtain a error probability with a significance of 0.5% .

To determine the efficiency of the plants to remove contaminants, all the aforementioned parameters were evaluated and contrasted with the contaminant concentration limits established by Article 7 in accordance with Res. 222/02, of the SEAM, currently the Ministry of Environment and Sustainable Development, of Paraguay.

## RESULTS

### Seeds propagation

In the September collection, **M1**, the spicules collected had greenish-yellow immature spikes and seeds did not detach from the rachis. From October, **M2**, spicules were yellow-brown and the seeds were detached with manual help. From November, **M3**, the already naturally dehiscent fruits, dropped seeds during transport, being yellow-brown the spicules color. From December, **M4**, the infructescences were still yellow-brown but seeds number had decreased due to their detachment from the rachis. Lastly, from January, **M5**, the spicules had a very low number of fruits.

**Table 1. Collected specimens description.**

| Lot | Collection date | Season | Spicule color | Germination (%) |
|---|---|---|---|---|
| $M_1$ | September 18 | Spring | greenish-yellow | 0 |
| $M_2$ | October 18 | Spring | yellow-brown | 2 |
| $M_3$ | November 18 | Spring | yellow-brown | 32 |
| $M_4$ | December 18 | Summer | yellow-brown | 72 |
| $M_5$ | January 18 | Summer | yellow-brown | Without fruits |

### Seed vigor estimation by accelerated aging method

Seed lot M4 was selected for the test, which showed the highest germination in optimal conditionsin the evaluation of germination in optimal conditions (72%). For aging test, the control treatment (without stress) had a slight increase in germination, having 74.33% of germination in optimal conditions, in aging treatments, it was decreasing, but the exposure treatment of seeds at 50 °C for 12 hours and 55°C for 24 hours were the ones that significantly decreased the germination percentage, registering values of 28% and 0.2%. Table 2 shows all values obtained in the accelerated seed aging test.

**Table 2. Description of collected specimens.**

| Treatment | Germination (%) | CV |
|---|---|---|
| Seeds without stress | 74.33 A | 9.72 |
| Seeds at 40 °C for 12 hours | 73.33 A | |
| Seeds at 45 °C for 24 hours | 67 A | |
| Seeds at 50 °C for 12 hours | 28 B | |
| Seeds at 55 °C for 24 hours | 0.5 C | |

**Means with a common letter are not significantly different ($p > 0.5$), CV: Coefficient of variation**

## Phytoremediation capability

To evaluate the household effluents purification efficiency, different parameters were measured *in situ*. Table 3 shows the results of these parameters: It can be seen that pH was decreasing. On day 1, it had a value of 6.2, but with the *Cyperus* plants treatment, pH decreased until having a 5.5 value by day 14. As for the temperature for both the control and the effluent with the plants, they maintained a temperature that ranged between 28 and 31 °C.

**Table 3. *In situ* parameter results.**

| Parameter | Day 1 | | Day 7 | | Day 14 | |
|---|---|---|---|---|---|---|
| | Control | Treated | Control | Treated | Control | Treated |
| **pH** | 6.2 | 6.2 | 6.2 | 5.8 | 6.0 | 5.5 |
| **Temperature(°C)** | 28 | 28 | 31 | 31 | 30 | 30 |
| **Dissolved oxygen(mg) $O_2$/L** | 0.81 | 0.80 | 0.69 | 2.23 | 0.35 | 2.45 |
| **Conductivity(µs)** | 585 | 587 | 580 | 210 | 567 | 245 |

The dissolved oxygen in the effluent at the beginning of the test was 0.81 mg $O_2$/L in the control, without the *Cyperus* plants. However, in the effluent treated with these plants it increased progressively until it reached a value of 2.45 mg $O_2$/L on day 14. Regarding conductivity, it began with records of 585 µs in control and 587 µs in treatment effluent, and a considerable reduction was seen too on day 14, registering 245 µs.

Regarding the parameters analyzed *ex situ*, there was a reduction in all parameters, as can be seen in Table 4. The biochemical oxygen demand (BOD) starting with a 375 mg $O_2$/L value, had a decrease on day 14 of 37 mg $O_2$/L, while the chemical oxygen demand, had an inicial value of 540 mg $O_2$/L, decreasing to 140 mg $O_2$/L.

**Table 4. *Ex situ* parameter results.**

| Parameter | Day 1 | | Day 7 | | Day 14 | |
|---|---|---|---|---|---|---|
| | Control | Treated | Control | Treated | Control | Treated |
| **DBO(mg $O_2$/L)** | 375 | 375 | 378 | 117 | 387 | 37 |
| **DQO(mg $O_2$/L)** | 540 | 540 | 587 | 289 | 592 | 140 |
| **Total Nitrogen (mg/L)** | 67 | 66 | 82 | 45 | 87 | 12 |
| **Total Phosphorus(mg/L)** | 5.5 | 5.5 | 5.5 | 4 | 5.5 | 3.8 |
| **Fecal Coliforms(CFU /100mL)** | 80,000 | 80,000 | 78,000 | 45,000 | 78,000 | 8,000 |

For Total Nitrogen, it can be seen it started with a value of 66 mg/L, and decreased to 12 mg/L on day 14. For Total Phosphorus, which initially registered a value of 5.5 mg/L, by day 14, treated by the plants, this parameter decreased to 3.8 mg/L. Regarding fecal coliforms, it initially had a 80,000 CFU/100mL value, but in treated effluent on day 14 it decreased to 8,000 CFU/100mL.

## DISCUSSION

### Propagation through seeds

Of the 5 species of *Cyperaceas* that **Martínez Peña *et al*., (2012)** were propagated, the percentage of germination of *Cyperus rufus* Kunth; *Eleocharis montana* (Kunth) Roem. & Schult. thaccord with those obtained in this research by exceeding more than 60% of germination.

The studies by Maas cited by **Schütz, (2000)**, show that germination percentages were significantly higher in four of six wetland species of the genus *Carex* after a period of six weeks of stratification. When analyzing the results, the germinative power increased substanciallyat 4 months of storage, in this way it could be considered that the *C. giganteus* presents a level of dormancy, however the germinative power after 5 months was decreasing to more than the half.

According to **Schütz& Rave, (1999),** dormancy and germination characteristics found in rushes are also important in other *Cyperaceae*. For example, the high level of latency is typical of most species of this family. Strict or conditional primary dormancy was found in annual species of *Cyperus* and *Fimbristylis* from North America, including species from dry sites (*Cyperus inflexus*), and from wetlands such as *Cyperus flavicomus, C. erythrorhizos, C. odoratus, Fimbristlis vahlii, F. autumnalis*, and agricultural weeds (*Cyperus esculentus* and *C. rotunda*). In this way, it could be considered that the seeds of *C. giganteus* have a type of dormancy.

The values obtained were higher than the *C. rufus*, *E. dombeya*, and *E. montana*species, which were 72%, 39%, and 75%, respectively, in the propagation protocol for hydrophiles proposed by **Martínez Peña *et al*., (2012)**.

## Estimation of seed vigor by the accelerated aging method

In this study, germination power, which was initially 74.33%, decreased notably after deterioration due to accelerated aging or produced large numbers of misshapen seedlings, which are considered to be of low vigor, thus indicating poor performance in stress conditions. The opposite occurs with lots of seeds with high germination under optimum conditions, which after accelerated aging does not greatly reduce the germination percentage and does not produce considerable amounts of abnormal seedlings, these seeds are considered to be of high vigor and with better ability for withstand stress conditions. **Hampton &Tekrony, (1995)** considers that those batches of seeds with a germination percentage greater than 80%, after accelerated aging, could be classified as high vigor, between 60 - 80% as medium vigor, and less than 60%, as low vigor. In this trial, the *Cyperus* seeds subjected to 40ºC for 12 hours practically maintained their germinative power, with only a difference of 2% less, with respect to the control (seeds without stress), obtaining 73.33% of germination, without statistical significance with respect to the control, as shown in **Table 2**. The batch of seeds subjected to 45ºC for 24 hours reduced its germination by 7%, with respect to the control, although there is a greater numerical difference, there is no statistical difference in the firstthree treatments. From the treatment that consisted of subjecting the seeds to 55°C for a time interval of 12 hours, the germination percentage decreased considerably, obtaining 28%, a percentage 47% lower than the control, this indicates that at this temperature in that range of time, the seeds begin to suffer considerable stress, causing the death of the embryos, probably due to the dehydration suffered. Considering that the seeds of this species are very small, with a very thin pericarp, thus facilitating a faster dehydration of the embryos. In the highest stress treatment, 55 °C for 24 hours, there was practically no germination, the deterioration was 100%, only 0.5% of the seeds germinated. According to **Delouche, (1976)**, deterioration has a negative connotation, while vigor has a very positive one, thus, vigor decreases as deterioration increases. Spoilage is the process of aging and death, and vigor is the main quality component affected by the spoilage process.

**Phytoremediation capability**

The results are encouraging and indicate that this species has bioremediation properties.The pH of the treated effluent of 6.2, had a decrease in 14 days of treatment with the plants, recording a value of 5.5 **(Table 3)**. The pH values obtained in this research are lower than those reported in a test with household effluents, with average values of pH between 7.39 and 7.42 pH, in a system using *T. domingensis* **(Jovania& Sandri, 2013)**. **Resolution 222/02 of Ministry of Environment and Sustainable Development (MADES)** of the Paraguay, establishes as an effluent discharge parameter that the pH value must be between 5 and 9, for which the values obtained in this work fall within the permitted ranges. Regarding dissolved oxygen (DO) values, an increase in DO concentration was obtained in waters treated with *C. giganteus* plants in contrast to the untreated effluent, with the values in the effluent showing that the plants have the ability to oxygenate the waters with oxygen from the atmosphere and deposite it through its roots that are in contact with the environment **(Ranalli & Lundholm, 2008)**.

The BOD recorded in the treatment of effluents with *C. giganteus* plants showed a substancial decrease in their values, this demonstrated the good performance of the plants, an indication of a high absorption of organic matter. Thus, the COD decreased and it is also an indicator of efficiency and retention of contaminants, in this case of the inorganic matter present in the effluent. These values obtained in the treated effluent were below the minimum values required by the SEAM. Thus, it is also important to characterize the type of effluent, taking into account the BOD/COD ratio, which makes it possible to determine how much COD (organic and inorganic matter contained in a sample) of a discharge is capable of being purified by microorganisms (BOD) and therefore the biodegradability of the different discharges. When the BOD/COD ratio is less than 0.3, the discharge is considered non-biodegradable, values between 0.3 and 0.7 make it slightly biodegradable, while values greater than 0.7 favor recovery conditions by biological methods according to **(Kemper & Mendoza, 2009)**.

Regarding the values of total Nitrogen and total Phosphorus, there was a fairly marked decrease in the treated water, all values below the minimum required by the MADES of the Paraguay, making clear the ability of contaminants retention by our biopurifying agent and the possibility of mitigating environmental damage in the final disposal of this type of effluent since the roots remain completely in contact with the medium. According to **Delgadillo *et al.*, (2010)** reports the purifying potential of wetlands varies seasonally and is directly proportional to the increase of temperature, therefore, at high temperatures, the capacity to remove phosphorus, phosphates and nitrogen is greater. It should also benoted that in effluents there are considerable amounts of nitrogen in the form of nitrites and ammonia nitrates. The decomposition and mineralization processes carried out by the microorganisms, transform that nitrogen to nitrites or nitrates (nitrification) and finally to nitrogen (denitrification). These processes largely depend on the concentration of oxygen in the system, which is thus absorbed by the plants in our treatment system, which take care of everything necessary for the absorption of the contaminants and its transformation needed to introduce them into the nitrogen cycle.

The values of total *coliforms* obtained in the treated effluent decreased in all of them, demonstrating the great removal of total *coliforms*, recording values around what is allowed by the MADES for final disposal of effluents. These results are similar to what was stated by **Kemper & Mendoza, (2009)**, that verified that the concentrations of *fecal coliforms* in wastewater were reduced from the first sampling and that it can reduce the levels in one or two logarithmicorders.

## CONCLUSION

With the present research study it was shown that the best time to collect *Cyperus giganteus* seeds for reproduction of plantains is the month of December, obtaining a percentage of seed germination of 72%.

The seeds vigor is moderate to progressively decreasing with temperatures above 50 ºC and time intervals over 12 hours in accelerated aging tests.

The species *Cyperus giganteus* has optimal properties to reduce the pollution load of household effluents.

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