

Research Article

Study of the Biomass of Macrophytes Presumed to Be Phytopurators in Wastewater Treatment in Brazzaville (Republic of Congo)

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Abstract

The aim of this work is to study the biomass of macrophytes during the wastewater phytoremediation process. To do this, a physicochemical characterization of these waters, including temperature, pH, EC, COD, NH_4^+ , NO_3^- , and PO_4^{3-} was carried out and first revealed the very high concentrations. Then, a treatment was done with two types of macrophytes (*Echinochloa pyramidalis* (Lam) Hutch and *Cyperus Alternifolius* L) which showed a significant reduction in these physicochemical parameters at the exit of the basins. When comparing the two treatment ponds, the *Echinochloa pyramidalis* pond performed better on the removal of pollutants from wastewater than the *Cyperus Alternifolius* pond. Finally, the study of the growth of these two types of macrophytes showed that *Echinochloa pyramidalis* grows faster than *Cyperus Alternifolius* and is almost double that of the latter. This study shows that the two macrophytes cannot be together in a filter to treat wastewater because the biomass of *Echinochloa pyramidalis* (Lam) Hutch is higher than that of *Cyperus Alternifolius* L and the two will compete against each other, *Echinochloa pyramidalis* (Lam) Hutch will hinder the growth of *Cyperus Alternifolius* L and lead to the gradual disappearance of the latter and this competition makes the macrophyte inefficient that seeks to defend its territory and gain space instead of eliminating pollutants.

Keywords

Phytopurification, Macrophytes, Growth, Wastewater

1. Introduction

Water is a precious asset for life, as a component of the global ecosystem. This resource, which meets basic human needs, is a development factor for generating and maintaining prosperity through human activities. However, these human activities generate huge amounts of wastewater that directly impacts ecosystems and people's health. Pollution in water is of industrial, domestic or agricultural origin [1]. In most countries

of the world, there is a growing interest in environmental protection, particularly wastewater management, both in terms of treatment and the installation of numerous sanitation facilities, given the considerable ecological impact that effluent discharges can have because water pollution is less and less tolerated. Indeed, it is estimated that only a third of the world's population (about 1.8 billion people) is served by a water

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treatment plant. The use of plants for wastewater treatment is a recent process that uses natural self-purification techniques and is receiving increasing attention [2]. This type of technology, which has been in operation for a decade, is quite cost-effective, easy to achieve with a much lower investment cost than a conventional wastewater treatment plant, contributes to the protection of the environment and human health, and can be applied to industrial, municipal or agricultural wastewater treatment [3]. The main pollutants that can be removed by such a system are nitrates, phosphates and heavy metals [4]. The combination of several macrophytes in the same filter to decontaminate wastewater is not very effective and leads to competition or the death of certain macrophytes present in the filter. In view of these difficulties encountered in phytoremediation, as a phenomenon of the gradual disappearance of certain macrophytes when combined with others, it is necessary to study the biomass of macrophytes during the process of phytoremediation of wastewater in order to understand their behaviour towards others.

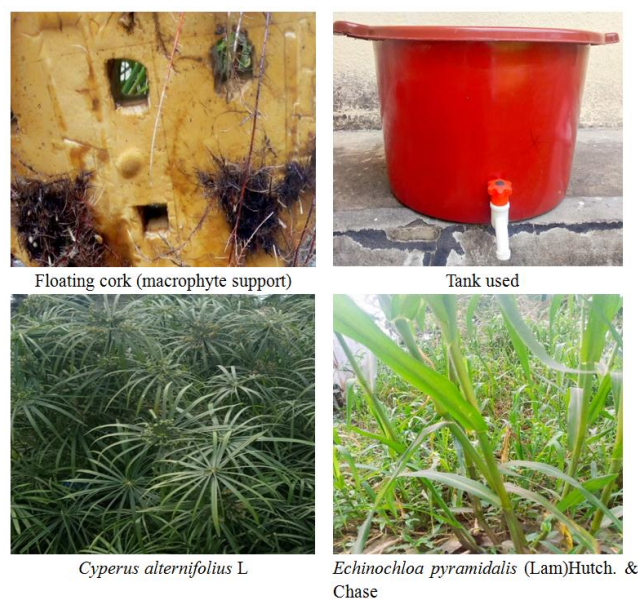


Figure 1. Experimental Filter Components.

2. Materials and Method

2.1. General Presentation of the Study Area

Brazzaville, the capital of the Republic of Congo, is located between 15 °20' and 15 °25' east longitude, and between 4 °10 and 4 °25' south latitudes. Its geographical position places it in the heart of Africa, therefore in the intertropical zone characterized by high temperatures and abundant rainfall. Indeed, Brazzaville records more than 1500 mm of water per year; this amount of water is unevenly distributed between two rainy seasons (October-November-December and March-April-May) [5].

2.2. Components of Experimental Filters

2.2.1. Plant Material

Two macrophytes were selected for this study: *Cyperus alternifolius* L and *Echinochloa pyramidalis* (Lam) Hutch. & Chase.

2.2.2. Tank Used

The tanks used for the experiments are made of plastic and have a maximum upper diameter of 40 cm and a lower diameter of 35 cm and a height of 30 cm. The floating support for attaching macrophytes is cork.

2.3. Experimental Design

The experimental device consists of 6 pilots (Figure 2) and a floating cork on which the macrophytes are attached, 3 of which are fixed *Echinochloa Pyramidalis* and the other 3 are fixed *Cyperus Alternifolius*, all fed with wastewater. In order to assess the effect of wastewater on macrophytes, a second macrophyte monitoring device has been developed in parallel with the same components and fed with tap water (ordinary water), and these macrophytes are taken as a control.



Figure 2. Experimental design during the study period.

2.4. Sampling Wastewater in Brazzaville

The water samples to be analysed were taken from shower drainage pits and gutters in homes and markets. The wastewater sampling campaign carried out in the various districts of Brazzaville is illustrated in Figure 3.



1) Wastewater sampling point

2) Wastewater sampling method

3) Wastewater in bottles

Figure 3. Wastewater sampling campaign in various districts of Brazzaville.

2.5. Physicochemical Parameter Analyses

2.5.1. Measurement of Hydrogen Potential and Temperature

The hydrogen potential (pH) and temperature (T) were measured by the potentiometric and thermometric method with a multi-parameter HI83141 by vertically immersing the electrode in a beaker containing at least 50 ml of the sample, then the reading was made after stabilization of the displayed value. (NF T 90-008) [6].

2.5.2. Electrical Conductivity (EC) Measurement

The conductivity was determined by vertically immersing the electrode of a conductivity meter (multi-parameter HI83141) in a beaker containing 50 ml of the sample and the reading is made after stabilization of the displayed value.

2.5.3. Turbidity Measurement

It was determined using a turbidity meter of the brand TURBIQUANT ORION AQ3010, by introducing at least 10 ml of the sample into a glass tank. The reading is done after the displayed value has stabilized.

2.5.4. Measurement of Suspended Solids (TSS)

They were determined using the filtration system method NFEN 872 (AFNOR, 2005), wastewater filtration, by first drying the filter paper in an oven at 105 °C and then weighing the mass of the paper (M_{empty}).

We filter 50 ml of the sample on the dried filter paper, we carry out the dehydration in the oven at 105 °C for 24 hours and then we weigh the paper again ($M_{105\text{ }^{\circ}\text{C}}$). The suspended solids content is calculated using the mass of dry matter obtained after separation of suspended solids from the wastewater, then dewatering at 105 °C in the oven. According to the formula:

$$\text{TSS} = (M_{105\text{ }^{\circ}\text{C}} - M_{\text{empty}}) / V_{\text{sample}}$$

TSS in mg/L; $M_{105\text{ }^{\circ}\text{C}}$ = constant mass of the sample after the oven (mg); M_{empty} = mass of the vacuum membrane (mg); V_{sample} = volume of the water sample studied (L).

2.5.5. Chemical Oxygen Demand (COD)

The chemical oxygen demand (COD) was measured by the oxidation method applied to the AFNOR standard (NF, T90-101) which consists of introducing 2 or 3 ml of sample into an acidic medium containing the appropriate reagent (commercial solution consisting of HgSO_4 , H_2SO_4 , Ag_2SO_4). The whole is heated in a COD oven (thermoreactor) at 150 °C for two hours (2 h). After cooling, the reading was taken using an AQUALYTIC-AL800 UV-visible spectrophotometer at a wavelength of $\lambda = 620\text{ nm}$.

2.5.6. Ammonium Ion Measurement

The ammonium ions (NH_4^+) were determined by the Nessler method (method 8038) which consists of taking 1 ml of the sample and adjusting it to 25 ml with distilled water. Three (03) drops of mineral stabilizer, 3 drops of polyvinyl alcohol are successively added and then stirred. 1 ml of Nessler's reagent is then added. After stirring the solution, the tank is introduced into the AQUALYTIC-AL800 brand UV-visible spectrophotometer. The reading was made at $\lambda = 425\text{ nm}$.

2.5.7. Nitrate Measurement

Nitrates (NO_3^-) were determined by dosing-calibration according to the NF T90-012 standard. Add 1 mL of sulphuric acid to each capsule. After 10 minutes, approximately 10 mL of water and 10 mL of ammonia are added to each capsule. The contents of each capsule are poured into a series of 25 mL volumetric flasks. Then, adjust to 25 mL with the rinse water from the capsule. Photometric measurements are made at the maximum of the absorption curve (wavelength around 415 nm)

after setting the device to zero absorbance with respect to water.

2.5.8. Orthophosphate Measurement

The concentration of orthophosphates (PO_4^{3-}) is determined by the dosage-calibration method. Introduce 20 ml of water into a 25 ml volumetric flask, then add 1 ml of ascorbic acid solution and continue as for establishing the calibration curve. Take into account the value read for the control. Refer to the calibration curve. The reading is taken with an AQUALYT-IC-AL800 UV-visible spectrophotometer at a wavelength $\lambda = 700$ nm or 880 nm.

2.6. Monitoring of Morphometric Parameters *Echinochloa Pyramidalis* and *Cyperus Alternifolius* During the Study Period

2.6.1. Length of Stems, Leaves, Roots and Diameters

The parameters considered for the monitoring are:

1. the size of the plants (stems) measured using a decimeter from the base of the rhizome to the level of the appearance of the young terminal leaves.
2. the length of the leaves and roots was measured using a calliper and a decimeter.
3. the diameter of the stems measured at 2 cm from the height of the rhizome using a calliper.

2.6.2. Water Content

Determination of fresh mass

After each macrophyte harvest on the two types of device (fed by wastewater and not), their fresh mass (Mf) was weighed using a Pinner PA512 balance.

After weighing the fresh mass (Mf), the samples of the collected plants were dried in the oven at a temperature of 105 °C for 24 hours, then weighed the dry mass (Ms).

The water content was determined by comparing the difference between the mass of the fresh matter and that of the dry matter over the mass of the fresh material.

$$\text{TE} = ((\text{Mf} - \text{Ms}) / \text{Mf}) * 100$$

With: TE: water content

Ms: Mass of Dry Matter

Mf: Mass of fresh material

3. Result and Discussion

3.1. Analysis of the Physico-chemical Parameters of Raw and Treated Wastewater

The results of the physico-chemical parameters are shown in Figures 4 to 9. They are the averages of each week.

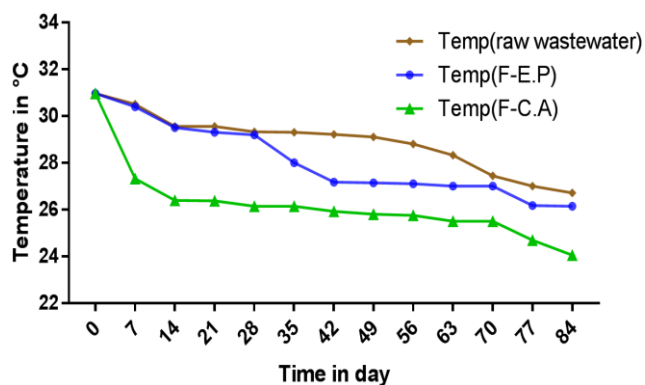


Figure 4. Temperature variation during treatment.

Temp: Temperature

F-C. A: *Cyperus Alternifolius* Filter

F-E. P: *Echinochloa Pyramidalis* Filter

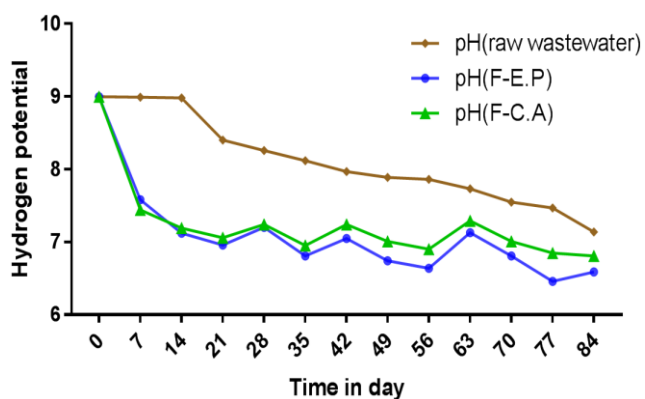


Figure 5. Variation in hydrogen potential (pH) during processing.

F-C. A: *Cyperus Alternifolius* Filter

F-E. P: *Echinochloa Pyramidalis* Filter

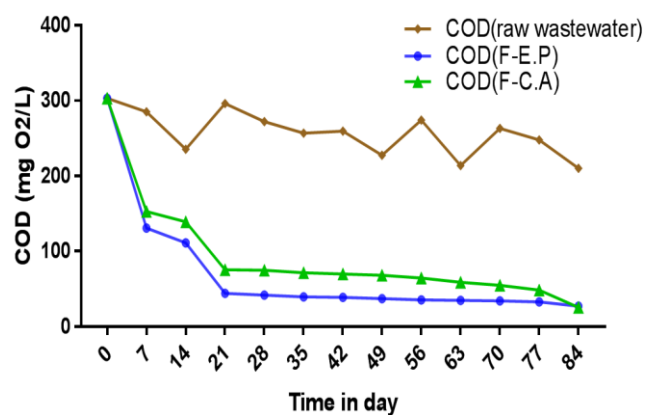


Figure 6. Variation in chemical oxygen demand (COD) during treatment.

F-C. A: *Cyperus Alternifolius* Filter

F-E. P: *Echinochloa Pyramidalis* Filter

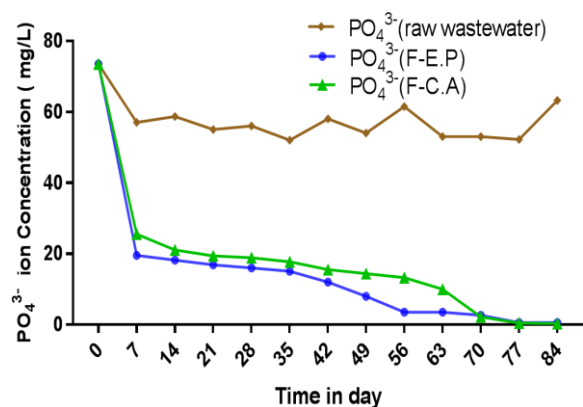


Figure 7. Variation in orthophosphates during treatment.

F-C. A: *Cyperus Alternifolius* Filter

F-E. P: *Echinochloa Pyramidalis* Filter

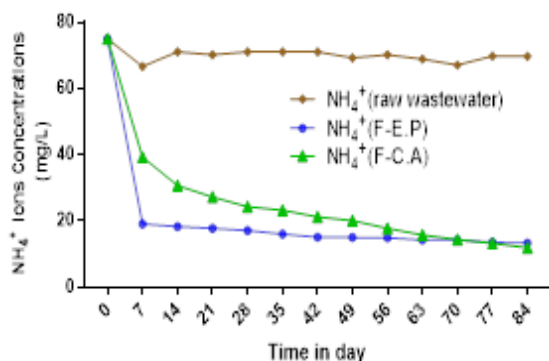


Figure 8. Variation in ammonium ions during treatment.

F-C. A: *Cyperus Alternifolius* Filter

F-E. P: *Echinochloa Pyramidalis* Filter

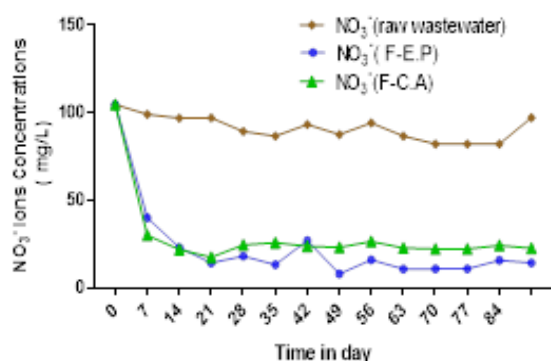


Figure 9. Variation in nitrates during treatment.

F-C. A: *Cyperus Alternifolius* Filter

F-E. P: *Echinochloa Pyramidalis* Filter

The temperature results for the wastewater feed and filtrates are shown in Figure 4. We note that the average temperature of

the feed wastewater is higher than that of the filtrates, varying around 28.78 °C, 27.84 °C and 25.80 °C respectively for the raw wastewater, *Echinochloa pyramidalis* and *Cyperus alternifolius*. The decrease in temperature in treated tanks may be due to a decrease in the number of bacteria. As for treated water from ponds *Echinochloa pyramidalis* et *Cyperus alternifolius*, they are different for the duration of the study period. Indeed, this difference in temperature can be explained by the two types of plants used and does not affect the life of the microorganisms responsible for purification. In general, the average values found during this study are favourable to the maintenance of colonies of purifying bacteria that develop at a temperature between 26-40 °C. Similar results have been found by some researchers who have used *Cyperus papyrus* as a purifying plant [7].

Regarding the variation of the hydrogen potential (Figure 5), the spectra show that the hydrogen potential of the feed wastewater is slightly neutral to alkaline with an average that fluctuates around 8.08 and decreases after each treatment with the average of 6.92 and 7.08 when *Echinochloa pyramidalis* and *Cyperus alternifolius* are used respectively. This decrease in hydrogen potential at the outlet of the treatment tanks can be explained by several processes that are more or less involved, in particular the oxidation of nitrites and COD, or by the metabolic activity of the root system of the plants used, which produces CO₂ [8]. The same observation was made by another author [9] who found a decrease in hydrogen potential from 7.2 in raw wastewater to about 6.6 to 7 in wastewater outlets from *Typha's* planted filters.

Chemical oxygen demand (COD) is a parameter that provides a clearer indication of the quantity of biodegradable and non-biodegradable organic matter contained in wastewater. Figure 6 shows the variation in this parameter and shows that the COD content of raw wastewater is higher than that of treated water. It varies around 254.99 mg O₂ /L in feed wastewater and decreases with each treatment, with the average varying around 50.87 mg O₂ /L and 75.32 mg O₂ /L respectively for *Echinochloa pyramidalis* and *Cyperus alternifolius*. This reduction in COD indicates that good bacterial assimilation takes place in the planted filters. This is probably linked to a better oxygenation of the latter, allowing aerobic bacteria to proliferate and consequently ensuring a better mineralization or oxidation of organic matter. These results are consistent with those of other authors [10].

The analysis of the average variation in the orthophosphate content (Figure 7) shows that the concentration of orthophosphates in the feed wastewater is higher than that of the treated water and has an average concentration of 57.43 mg/L; 9.73 mg/L and 13.27 mg/L respectively for raw wastewater, *Echinochloa pyramidalis* and *Cyperus alternifolius*. The elimination of orthophosphates in the treatment ponds can be involved in the consumption of the plants for their physiological needs [11]. At the same time, microbial populations that reside in submerged roots can assimilate PO₄³⁻ ions from wastewater [12].

From Figure 8, we can see that the ammonium ion content in treated wastewater is remarkably lower than that of feed

wastewater and has an average content of 69.71 mg/L, 15.72 mg/L and 21.60 mg/L respectively for raw water, *Echinochloa pyramidalis* and *Cyperus alternifolius*. The lowest values are observed at the *Echinochloa pyramidalis* followed by *Cyperus alternifolius*. These results can be explained by the decomposition of the organic matter that gives ammonium (ammonification) and the latter is transformed into ammonia (volatilization). These results corroborate with several authors [13]. Indeed, aquatic macrophytes are endowed with a well-developed internal air space (aerenchyma) to ensure the transfer of oxygen to the roots and rhizomes which transform ammonium ions into nitrates or nitrite [14].

According to the curves (Figure 9), the nitrate content at the outlet of the treatment ponds is lower than that of the feed wastewater. The average nitrate concentrations in the treated water fluctuate around 15.20 mg/L and 23.12 mg/L respectively for *Echinochloa pyramidalis* and *Cyperus alternifolius*, compared to 90.26 mg/L for the feed wastewater. This reduction in nitrates for purified water can be explained by their consumption by plants. The same observation has been made by another author [15], who used *Phragmites australis* as a purifying plant.

3.2. Monitoring of *Echinochloa Pyramidalis* and *Cyperus Alternifolius* Morphometric Parameters During the Study Period

The variation in stem lengths is shown in Figure 10. We notice that the sizes of the stems gradually increase over time and reach averages around 3.37 m for *Echinochloa pyramidalis* and 2.08 m for *Cyperus Alternifolius*. These sizes reach maximums of 5.01 m and 3.13 m respectively for *Echinochloa pyramidalis* and *Cyperus Alternifolius* after treatment with wastewater. However, the sizes are smaller before treatment (0.63 m and 0.53 m as a maximum) respectively for *Echinochloa pyramidalis* and *Cyperus Alternifolius*. In general, *Echinochloa pyramidalis* and *Cyperus Alternifolius* seem to be well adapted to wastewater ponds, their growth is exponential. Indeed, the rapid growth of macrophytes after treatment with wastewater is due to a sufficient supply of organic matter and nutrients present in the feed wastewater [16, 17]. However, it is important to note that *Echinochloa pyramidalis* grows faster than *Cyperus Alternifolius*.

Figure 11 shows that wastewater significantly modifies the biomass of the groundwater (roots) of macrophytes after treatment with wastewater, unlike the roots of macrophytes before treatment with wastewater. In fact, the root lengths of macrophytes after treatment with wastewater reach maximums of 38.00 cm for *Echinochloa pyramidalis* and 24.00 cm for *Cyperus Alternifolius*, in contrast to the smallest sizes obtained. In general, the root lengths of macrophytes after treatment with wastewater are very small compared to macrophytes after treatment with wastewater. Our results are consistent with those of the other authors [16, 18] who have observed an increase in biomass in forage plants when they are irrigated with raw wastewater.

The analysis in Figure 12 shows that the leaf lengths of macrophytes after treatment with wastewater are much greater than

those found before treatment with wastewater. These lengths increase exponentially with an average of 51.46 cm for *Echinochloa pyramidalis* and 21.67 cm for *Cyperus Alternifolius*, and reach maximums of 61.00 cm and 35.00 cm respectively *Echinochloa pyramidalis* and *Cyperus Alternifolius* after treatment with wastewater. Unlike the smaller sizes obtained before treatment with wastewater (12.20 cm and 7.00 cm as maximums) for *Echinochloa pyramidalis* and *Cyperus Alternifolius*. In general, the leaf lengths of macrophytes before treatment with wastewater are reduced by half compared to macrophytes after treatment with wastewater. Biomass production is higher in plants watered by raw wastewater than in controls. Watering with raw wastewater implies better assimilation due to nutrient inputs, in particular nitrogen compounds which induce significant photosynthetic production in the leaves [17, 19].

The analysis of this figure 13 shows that these diameters have an average of 6.79 mm for *Echinochloa pyramidalis* and 3.85 mm for *Cyperus Alternifolius*, after treatment with wastewater and reach maximums of 12.25 and 8.90 mm under the same conditions. Regarding the diameters of the stems of the macrophytes before treatment with wastewater, they are smaller with, as maximums of 7.25 and 3.45 mm respectively *Echinochloa pyramidalis* and *Cyperus Alternifolius*. These results are lower than those of Hardej and al [20], which mention a small variation in the diameter of *Phragmites australis* fed by sludge from domestic wastewater treatment plants.

Figure 14 illustrates the average variation in the fresh and dry mass before and after the passage of raw wastewater through the two planted filters. There is a gradual increase over time in the fresh masses of two macrophytes, the average values of fresh masses are 68.75 g for *Echinochloa pyramidalis* before treatment and increases to 132.06 g on the 30th day and 142.96 g after 90 days after wastewater treatment. For *Cyperus alternifolius*, it is 12.71 g before treatment and increases to 20.32 g on the 30th day and 30.49 g after 90 days after wastewater treatment. As for dry masses, it is noted that these masses evolve in the same way over time with an average value of 7.87 g for *Echinochloa pyramidalis* before treatment and increases to 42.26 g on the 30th day and 41.46 g after 90 days after wastewater treatment. For *Cyperus alternifolius*, it is 1.35 g before treatment and increases to 5.28 g on the 30th day and 7.93 g after 90 days after wastewater treatment. In general, the two masses (fresh and dry) of macrophytes before treatment are much lower than the masses of macrophytes after treatment with wastewater. This difference may be due to the nutrients or organic matter provided by the wastewater.

Concerning the content (Figure 15), there is a gradual decrease over time in the water content of two macrophytes, the average values of the water content is 88.51% for *Echinochloa pyramidalis* before treatment and increases to 68.00% on the 30th day and 71.00% after 90 days after wastewater treatment. For *Cyperus alternifolius*, it is 89.47% before treatment and increases to 74.02% on day 30 and 73.99% after 90 days after wastewater treatment. The high water content observed in plants before treatment could be justified by the lack of nutrients in ordinary water and plants are forced to absorb enough to com-

compensate for this deficit. Thus, these results are similar to those of Tsama and al [21].

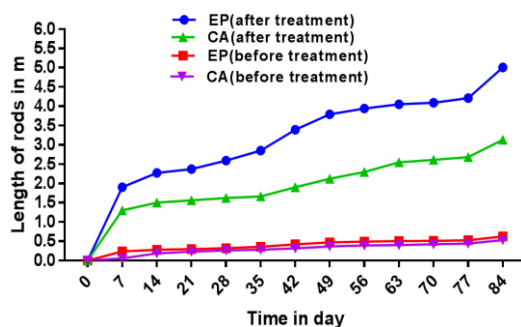


Figure 10. Length of rods during treatment.

E. P: *Echinochloa Pyramidalis*
C. A: *Cyperus Alternifolius*

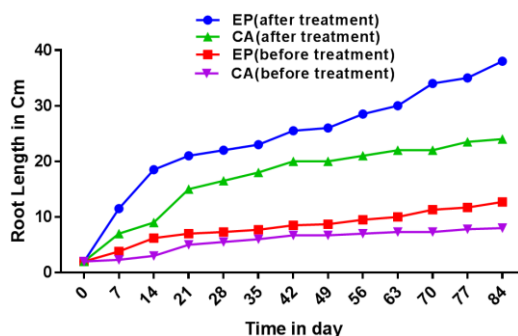


Figure 11. Root length during treatment.

E. P: *Echinochloa Pyramidalis*
C. A: *Cyperus Alternifolius*

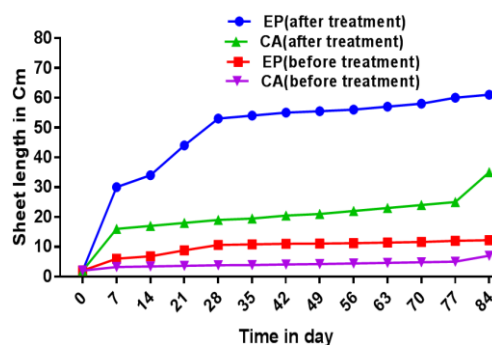


Figure 12. Leaf length during processing.

E. P: *Echinochloa Pyramidalis*
C. A: *Cyperus Alternifolius*

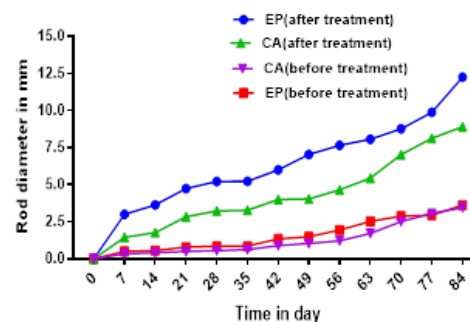
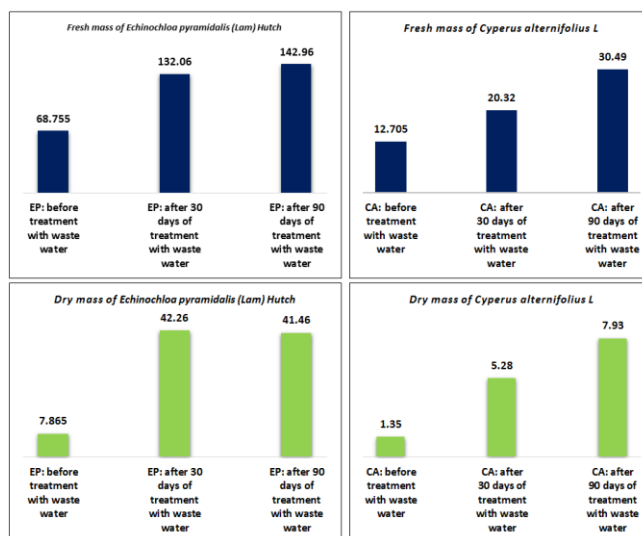


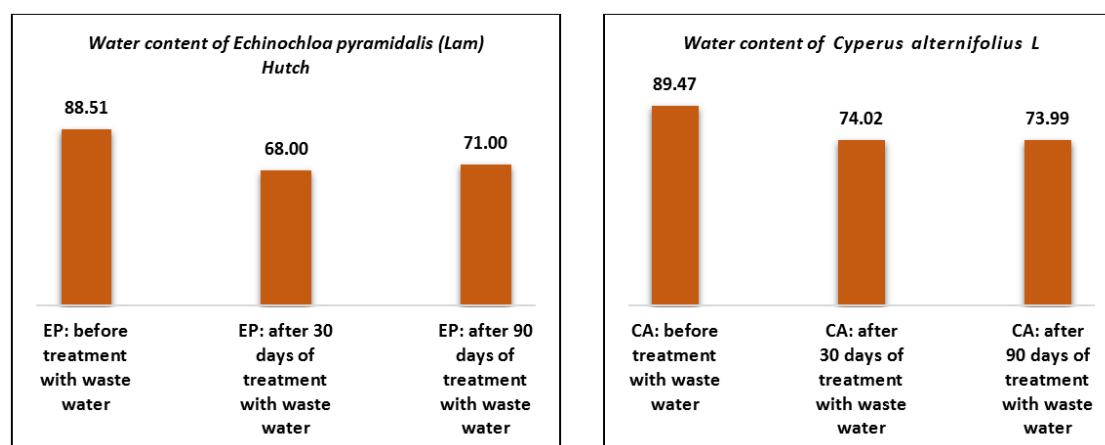
Figure 13. Rod diameter during treatment.

E. P: *Echinochloa Pyramidalis*
C. A: *Cyperus Alternifolius*



EP: *Echinochloa pyramidalis* CA: *Cyperus alternifolius* L.

Figure 14. Variation in the fresh and dry mass of the two macrophytes used.



EP: *Echinochloa pyramidalis* CA: *Cyperus alternifolius*

Figure 15. Variation in the water content of the two macrophytes used.

4. Conclusion

The aim of this work was to study the growth of macrophytes during the wastewater phytoremediation process. Analysis of the physicochemical parameters of the wastewater revealed very high concentrations of pollutants. However, during the phytoremediation process, the results obtained showed:

1. A slight decrease in hydrogen potential (pH), temperature and electrical conductivity for the two *Echinochloa pyramidalis* and *Cyperus alternifolius* ponds.
2. A very significant reduction in chemical oxygen demand of around 50.87 mg O₂ /L for the *Echinochloa pyramidalis* pond and 75.32 mg O₂ /L for the *Cyperus alternifolius* pond.
3. A very clear reduction of the order of 9.73 ± 6.57 ; 15.72 mg/l and 15.20 mg/L ortho phosphates, ammonium ions and nitrates respectively when *Echinochloa pyramidalis* is used. For *Cyperus alternifolius*, the order is 13.27 mg/l; 21.60 mg/l and 23.12 mg/l ortho phosphates, ammonium ions and nitrates respectively.

Overall, wastewater influences the growth and development of *Echinochloa pyramidalis* and *Cyperus alternifolius*. Indeed, *Echinochloa pyramidalis* reached the maximum values of 2.51 m, 3.80 cm, 12.20 cm and 7.25 mm respectively in size, roots, leaves and diameter. On the other hand, *Cyperus alternifolius* reached the maximum values of 1.57 m, 2.30 cm, 7.00 cm and 6.90 mm respectively for the same parts of the plant. It should be noted that the said plants.

Finally, this work allowed us to observe that macrophytes subjected to wastewater grow faster than those subjected to ordinary water. In general, under the same conditions, the growth and development of *Echinochloa pyramidalis* is higher compared to *Cyperus alternifolius*.

At the end of this study the two macrophytes cannot be together in a filter for the treatment of raw wastewater, the

two will compete against each other because given their evolution (growth and biomass) of the *Echinochloa pyramidalis* (Lam) Hutch it will hinder the growth of *Cyperus Alternifolius* L and will lead to the gradual disappearance of the latter and this competition will lead to the inefficiency of the dominant macrophyte which seeks To defend its territory and gain space instead of eliminating pollutants.

Abbreviations

E. P	<i>Echinochloa Pyramidalis</i>
C. A	<i>Cyperus Alternifolius</i>
F-C. A	<i>Cyperus Alternifolius</i> Filter
F-E. P	<i>Echinochloa Pyramidalis</i> Filter
M	Meter
Cm	Centimeter
TSS	Suspended Solids
mg/l	Milligram Per Liter
mm	Millimeter
EC	Electrical Conductivity
COD	Chemical Oxygen Demand

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Conflicts of Interest

The authors declare that no conflicts of interests.

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