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Characterization of the chemical composition of *Pontederia crassipes* from the Casa Blanca lagoon and Coatzacoalcos river in the state of Veracruz (Mexico)

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A B S T R A C T

Water lily (*Pontederia crassipes*) is a common hydrophytic plant present in many water bodies, however, its potential is underestimated in various applications. This study aimed to characterize their chemical composition and morphology, as well as to explore their possible sustainable applications. Consequently, water lily samples were collected and FTIR and SEM analyses were performed to understand its chemical and morphological structure. The results revealed that water lily is rich in cellulose and lignin, which makes it ideal for the removal of contaminants and various biotechnological applications. In addition, its possibilities in the production of biodegradable materials, bioplastics and biofuels are highlighted. Although the influence of geographical variations in its composition is recognized, this study lays the foundation for further research and the development of sustainable technologies that may take advantage of this underestimated resource. Its implications range from solving environmental problems to benefiting society and industry, promoting valuable and sustainable solutions in a global context.

Keywords: Biomass, Functional groups, Biomaterials.

Caracterização da composição química de *Pontederia crassipes* da lagoa Casa Blanca e do rio Coatzacoalcos, no estado de Veracruz (México)

R E S U M O

A planta aquática lírio-d'água (*Pontederia crassipes*) é comum em corpos d'água e tem um potencial subestimado em várias aplicações. Este estudo teve como objetivo caracterizar sua composição química e morfologia, bem como explorar suas possíveis aplicações sustentáveis. Foram coletadas amostras de lírio-d'água e foram realizadas análises FTIR e SEM para compreender sua estrutura química e morfológica. Os resultados revelaram que o lírio-d'água é rico em celulose e lignina, tornando-o adequado para a remoção de contaminantes e diversas aplicações biotecnológicas. Além disso, destacam-se suas possibilidades na produção de materiais biodegradáveis, bioplásticos e biocombustíveis. Embora seja reconhecida a influência das variações geográficas em sua composição, este estudo estabelece as bases para futuras pesquisas e desenvolvimento de tecnologias sustentáveis que aproveitem este recurso subestimado. Suas implicações abrangem desde a resolução de problemas ambientais até o benefício da sociedade e da indústria, promovendo soluções valiosas em um contexto global.

Palavras-Chaves: Biomassa, Grupos Funcionais, Biomateriais.

Caracterización de la composición química de *Pontederia crassipes* de la laguna Casa blanca y río Coatzacoalcos del estado de Veracruz (Mexico)

R E S U M E N

El lirio acuático (*Pontederia crassipes*) es una planta hidrófita común en cuerpos de agua que presenta un potencial subestimado en diversas aplicaciones. Este estudio tuvo como objetivo caracterizar su composición química y morfología, así como explorar sus

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posibles aplicaciones sostenibles. Se recolectaron muestras de lirio acuático y se realizaron análisis FTIR y SEM para comprender su estructura química y morfológica. Los resultados revelaron que el lirio acuático es rico en celulosa y lignina, lo que lo hace idóneo para la eliminación de contaminantes y diversas aplicaciones biotecnológicas. Además, se destacan sus posibilidades en la producción de materiales biodegradables, bioplásticos y biocombustibles. Aunque se reconoce la influencia de las variaciones geográficas en su composición, este estudio sienta las bases para futuras investigaciones y el desarrollo de tecnologías sostenibles que aprovechen este recurso subestimado. Sus implicaciones abarcan desde la resolución de problemas ambientales hasta el beneficio de la sociedad y la industria, promoviendo soluciones valiosas en un contexto global.

Palabras clave: Biomasa, Grupos funcionales, Biomateriales.

1. Introduction

Water lily is a free, floating, and hydrophytic plant that belongs to the *Pontederiaceae* family (INECOL, 2023). It is known scientifically as *Pontederia crassipes* and is characterized by its beauty and rapid adaptation to the aquatic environment (EPPO, 2023; IPNI; 2023; POWO, n.d.). It is a perennial plant commonly found in ponds, lakes, and other bodies of water. It has a unique structure consisting of floating leaves on the surface of the water and roots anchoring in the bed of the body of water (INECOL, 2023).

The leaves of the water lily are large and round, with a heart-shaped appearance. They have a deep green color and often have a waxy coating that helps repel water. These floating leaves allow it to capture the sunlight needed to perform photosynthesis. Water lily flowers are one of its most distinctive and attractive features. As for its adaptation to the aquatic environment, *Pontederia crassipes* has long, branching roots that extend at the bottom to obtain nutrients and water. In addition, these roots help keep the plant anchored and stable in its aquatic environment.

As pointed out by Tovar-Jiménez in his 2019 study, water lily has a composition ranging from 92.8 % to 95 % water content, volatile compounds ranging from 4.2 % to 6.1 %, 18.2 % to 19 % cellulose, hemicellulose ranging from 48.7 % to 50 %, 3.5 % to 3.8 % lignin and from 13 % to 13.5 % crude protein. These figures suggest its versatility for various applications; however, these concentrations can fluctuate depending on geographical location and climatic factors.

This was demonstrated in Mexico, in different regions (Mexico City, Hidalgo, Jalisco and Tabasco) where it was found that the ash content, extractable in water, in solvent and holocellulose content present statistical differences between the states of the republic. To this respect, Tovar-Jiménez (2019, p. 50) concluded that "the samples from Tabasco had the highest concentration of ash, those from Hidalgo the highest concentration of lignin and those from Jalisco the highest percentages of extractable compounds in water. On the other hand, holocellulose showed the greatest variability with minimal significant differences, both at the level of the collection area and the plant fraction".

The holocellulose content (cellulose + hemicellulose) had less variation 28 % to 35 %. Regarding the ash content, the samples from Mexico City, Hidalgo and Tabasco presented, on average, 16.16, 17.56 and 19.8 % (Tovar-Jiménez, 2019) in contrast to that published by Gao et al. (2013), who report an ash content of 45 % and Ma et al. (2010) of 2.8 %, both of lily collected in China, which may be due to climatic differences, age of the plant, altitude, geographical area, among other factors. The holocellulose content is important due to the carbohydrates present in it that have the potential to be used in a wide variety of biotechnological processes, such as bioethanol production (Guragain et al., 2011; Nigam, 2002).

Currently, it is classified as a weed, because through its rapid multiplication it prevents the free passage of the sun and oxygen, affecting the survival of animals and plants that share its ecosystem (Kriticos & Brunel, 2016). There is evidence that it negatively impacts the health of the population, water supply, economic activities such as fishing, and agricultural production (Villamagna & Murphy, 2010; Enyew, Assefa & Gezie, 2020). Its eradication has not been effective, it is commonly extracted from water bodies and piled up outside them, hoping to die naturally. In some countries such as Ethiopia, Mexico and Australia, machinery has been invested in its crushing and control bioagents have been introduced, nevertheless, these efforts have failed

(Abbott et al., 2020; Bonilla-Barbosa & Santamaría, 2014; Enyew, Assefa & Gezie, 2020). Thus, it is necessary to seek the sustainable use of this species in order to balance its reproduction. In some documented research, water lily has been proposed as a phytoremediator for the extraction of heavy metals and wastewater (Agunbiade, Olu-Owolabi & Adebowale, 2009; Salamah, Perwira & Kurniawan, 2019; Ilham, Nafie & Budi, 2020; Adewoye, Adenigba & Adewoye 2021; Auchterlonie, Eden & Sheridan, 2021; Prasad et al., 2021); food for fish, mudworms and goats (Ikhsan et al., 2021; Isnawati & Trimulyono, 2018; Safitri et al., 2021; Saviolo et al., 2020); production of a biocomposite comprising water lily and starch (Syafri et al., 2019; Zambrano et al., 2022); and water lily cellulose insulation to be used as a reinforcing component in the production of bioplastics (Asrofi et al., 2018; Pratama, et al., 2020).

It is believed that water lily arrived in Mexico through intentional or accidental introduction by humans. There are several theories as to how it could have been introduced into the country. Some possible routes of entry include: 1) Ornamental introduction: It is possible that the water lily has been imported as an ornamental plant for ponds and water gardens (MONGABAY, 2022; Rodríguez-Lara, et al., 2022) and 2) Accidental contamination: Another possibility is that the water lily has been introduced through accidental contamination of water bodies. Its seeds or vegetative fragments could have been transported along with boats, fishing equipment, irrigation canals or other means, and subsequently released into Mexican bodies of water (Miranda & Lot, 1999). Once established in Mexico, water lily has proven to be an invasive species (CONABIO, 2010), capable of spreading rapidly and forming dense colonies in various bodies of water. This has generated concern due to its negative effects on native aquatic ecosystems and on the human uses of such bodies of water, like navigation and water supply.

Water lily (*Pontederia crassipes*) is a species whose importance has been underestimated, despite its presence in water bodies around the world. Thus, this research seeks to characterize it and provide proposals to address the complex environmental challenges that its proliferation causes, as well as to offer solutions that may benefit both nature and society.

2. Material and Methods

2.1 Study Area

The Casa Blanca Lagoon is located in the municipality of Xalapa, Veracruz, Mexico. It serves as a wetland for migratory birds. Locals have attempted to develop it as a tourist attraction; however, the body of water has not been able to be utilized for this purpose due to a lack of economic resources and political will in the area (Salazar, 2023).

The collection of water lily was carried out in the Casa Blanca lagoon, municipality of Xalapa and the Coatzacoalcos river in Nanchital, Veracruz, Mexico. These study areas were chosen for comparative analysis of their pollution leves.

For this study, the roots were discarded, using only the stem and leaves. Subsequently, successive washes were carried out with drinking water and then with deionized water to remove impurities (Figure 1, 2). Moreover, a drying process was carried out in an oven at a constant temperature of 180° C for 12 hours. Finally, the dried biomass was crushed in a mill and sieved with an ASTM No. 50.

Figure 1 - Water lily leaves
Figura 1 – Folhas de lírio d'água

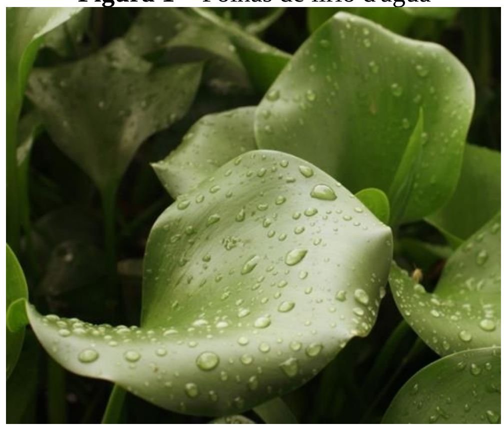
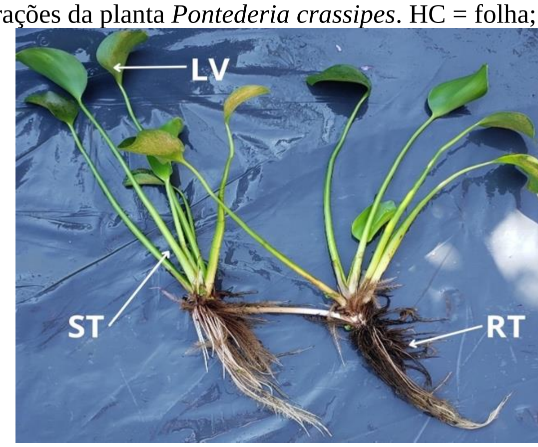


Figure 2 - Fractions of the *Pontederia crassipes* plant. LV = leaves; ST = stem; RT = roots
Figura 2 – Frações da planta *Pontederia crassipes*. HC = folha; TA = caule; RA = raízes



The Coatzacoalcos River is situated in the southeastern part of the Mexican Republic. It originates in the state of Oaxaca in the Niltepec mountain range and flows into the Gulf of Mexico. It is one of the most voluminous rivers in Mexico, considered the second most important in the state of Veracruz. It reaches depths of up to 15 meters and is navigable for two-thirds of its course (INEGI, 2016). The municipalities of Coatzacoalcos and Nanchital de Lázaro Cárdenas del Río are located in the Mexican Isthmus, in the southern part of the state of Veracruz-Mexico, in the region known as the Industrial Corridor - Uxpanapa.

The corridor is a home to one of Mexico main industrial zones, housing several of the country's most important petrochemical complexes (Espinosa-Reyes, 2009). The Coatzacoalcos River is considered by several authors as one of the sites with the greatest environmental contamination problems in Mexico (Albert et al., 2005; Espinoza-Reyes, 2009; Ilizaliturri, 2010).

2.2 Solid-liquid extraction

Once the crushed biomass was obtained, the solid-liquid extraction process was carried out using the technique of Figueroa and Martínez (2020). A 2 g lily sample was placed on a dry basis in a conical filter previously dried in the oven for 30 minutes. Then, the sample was introduced into the equipment for 4 hours at 80° C. The extraction was carried out with Methanol 1 ½ times for 4 hours until the solution was colorless.

2.3 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR Spectroscopy was performed to understand the chemical structure of water lily (*Pontederia crassipes*). It was carried out at the Center for the Development of Biotic Products (CEPROBI) of the National Polytechnic Institute. Functional groups in the water lily were identified by Fourier transform infrared spectroscopy (FTIR) on a Shimadzu IR Affinity spectrophotometer. For the analysis of each sample, 0,002 g of dried water lily biomass was used. It was analyzed in transmittance mode with attenuated total reflectance (ATR) in the mid-range region of 650 to 4000 cm-1 with a resolution of 4 cm-1 and 60 scans.

2.4 Scanning Electron Microscope (SEM)

The morphological analysis and determination of the elemental composition of (*Pontederia crassipes*) was carried out on a scanning electron microscope brand Carl Zeis (EVO LS10, Germany) with an acceleration voltage of 30 kV. Three water lily samples were used: a) powdered treated lily, b) lily leaf and stem from the Coatzacoalcos River, and c) lily leaf and stem from the Casa Blanca lagoon, Xalapa, Veracruz, Mexico. The samples were placed on aluminum beads with double-sided carbon conductive tape and observed in the MEB at a constant pressure of 50 Pa with nitrogen gas, a backscattered electron detector, and an X-ray detector (Bruker, Quantax 200, Germany).

3. Results and Discussion

The functional groups that are present were determined by FTIR spectrum analysis. Figure 3 shows the infrared spectrum obtained for the dried water lily biomass and Figure 4 for the treated biomass.

Figure 3 - FTIR spectrum of the dry biomass of *Pontederia crassipes*.
Figura 3 – Espectro FTIR da biomassa seca de *Pontederia crassipes*

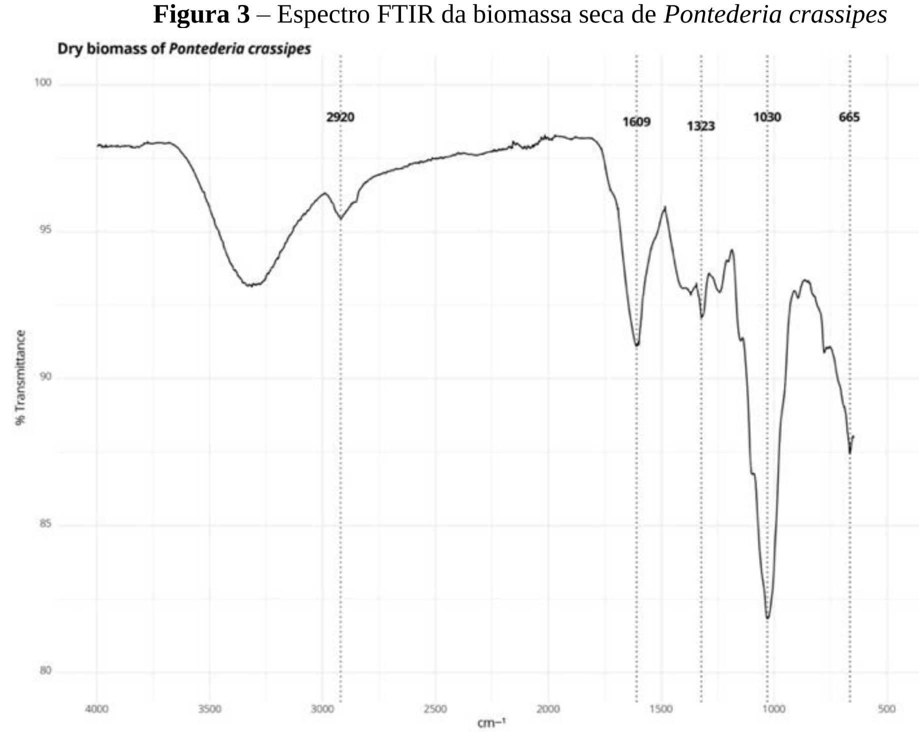
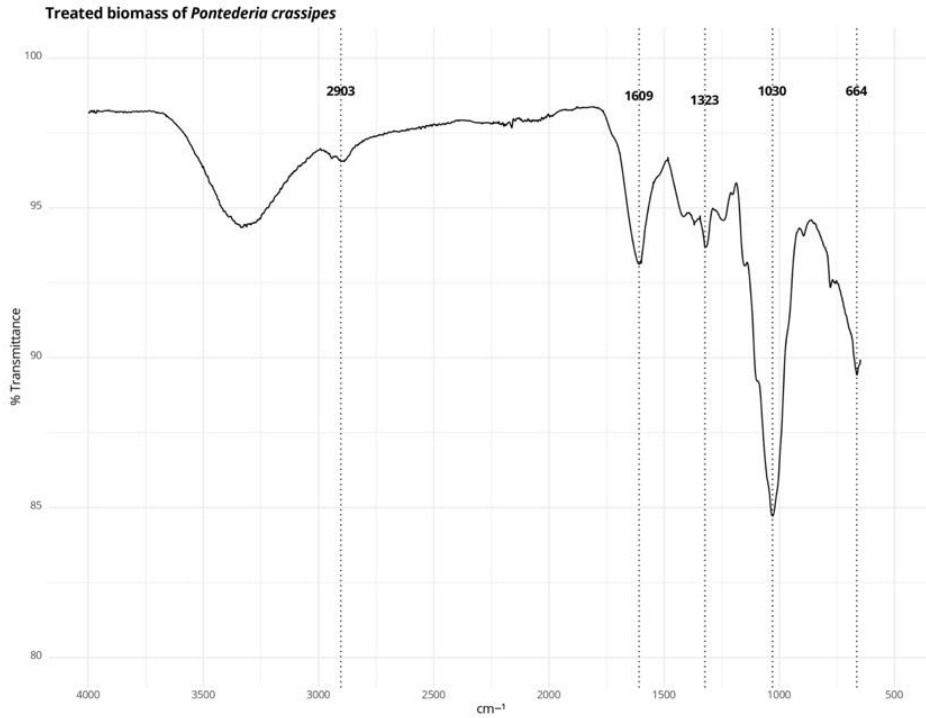


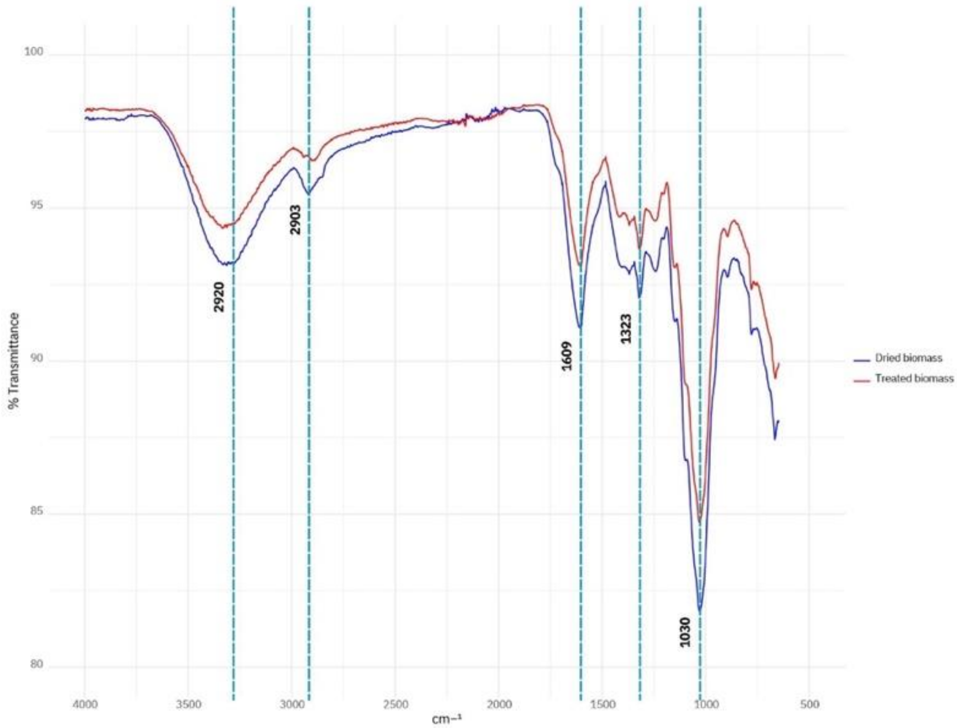
Figure 4 - FTIR spectrum of the treated biomass of *Pontederia crassipes*
Figura 4 – Espectro FTIR da biomassa tratada de *Pontederia crassipes*



In both, the broad band between the lengths of 3000 to 3600 cm–1 can be observed. In this region, vibrations of the –OH group appear, the most abundant in cellulose and amino groups (Lavado-Meza et al., 2020). The peak at 2920 and 2903 cm–1 respectively correspond to the asymmetric vibration of the C–H bond specifically aliphatic C-H bonds (Shahrokhi-Shahraki et al., 2021). The band at 2200 cm-1 is attributed to the deformation of CO, in which the vibrations of the carbonyl groups (C=O) from esters, amides and, to a lesser extent, carboxylic acids are considered. At 1609 cm 1, it is caused by the vibrations of the aromatic C=C bond. The band centered at 1323 cm–1 is the narrow vibration of a dimer carboxylic acid that may be present in the cellulose. Finally, a strong peak at 1030 cm–1 corresponds to the vibration of the -C-O bond. The obtained water lily dry biomass spectrum was similar to that reported by Komy et al., (2013) and Parameswari et al., (2021).

The FTIR spectra of dry biomass and treated biomass of water lily are compared to show the presence of various functional groups such as C-O at 1030 and 1249 cm-1; N-H at 1323 cm-1; C-H at 2920 cm-1; characteristic of pectin, cellulose, hemicellulose and lignin, which reflects a complex chemical composition due to the presence of these functional groups (Martinez-Vasquez et al., 2020) (Figure 5).

Figure 5 - Comparison of FTIR spectra of dried and treated biomass of *Pontederia crassipes*
Figura 5 – Comparação dos espectros de FTIR da biomassa seca e tratada de *Pontederia crassipes*

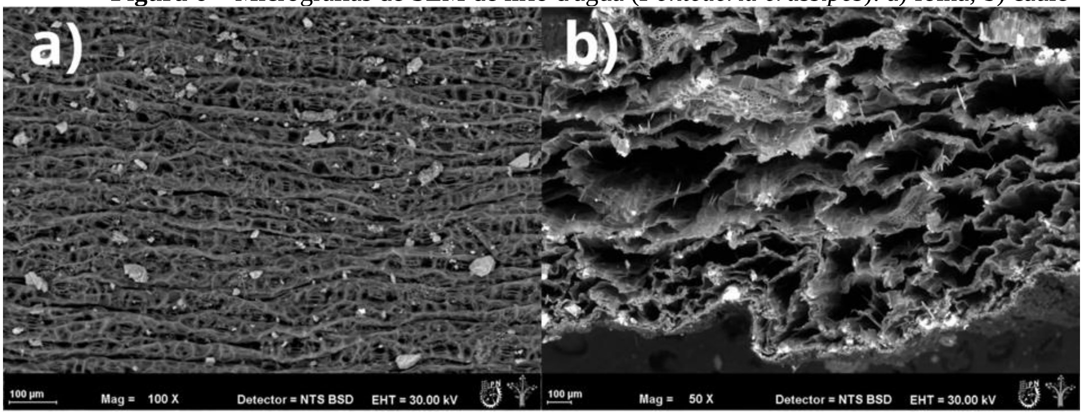


The difference between dry biomass and treated biomass can be attributed to the increase in absorption capacity due to the chemical modification of the raw material and the composition of cellulose, hemicellulose, lignin, crude ash, etc., as well as structural characteristics such as the crystallinity index of cellulose (Priya & Selvan, 2014; Feng et al., 2017).

The hydroxyl (OH), carbonyl (C=O), C–O–C ethers functional groups in aromatic groups and aromatic amines in the lignocellulosic structure of the water lily gives it the ability to interact with heavy metals and provide multiple sites for the adsorption of Cr (III and VI), Cu, Pb and Hg (Komy et al., 2013; Wei et al., 2017; Tejada-Tovar et al., 2021; Ardila-Arias et al., 2022). Similarly, to take advantage of its high fiber content, dried or treated water lily biomass can be converted into a food supplement, substrate or nanoparticles and can be used as a natural packaging for filters or as a water-absorbing material. Hydrolyzed biomass has been successfully used as carbon to produce poly ß-hydroxybutyrate (PHB) for the pharmaceutical, medical, food and agricultural industries as it has properties similar to synthetic plastics, however, they are biodegradable (Feng et al., 2017).

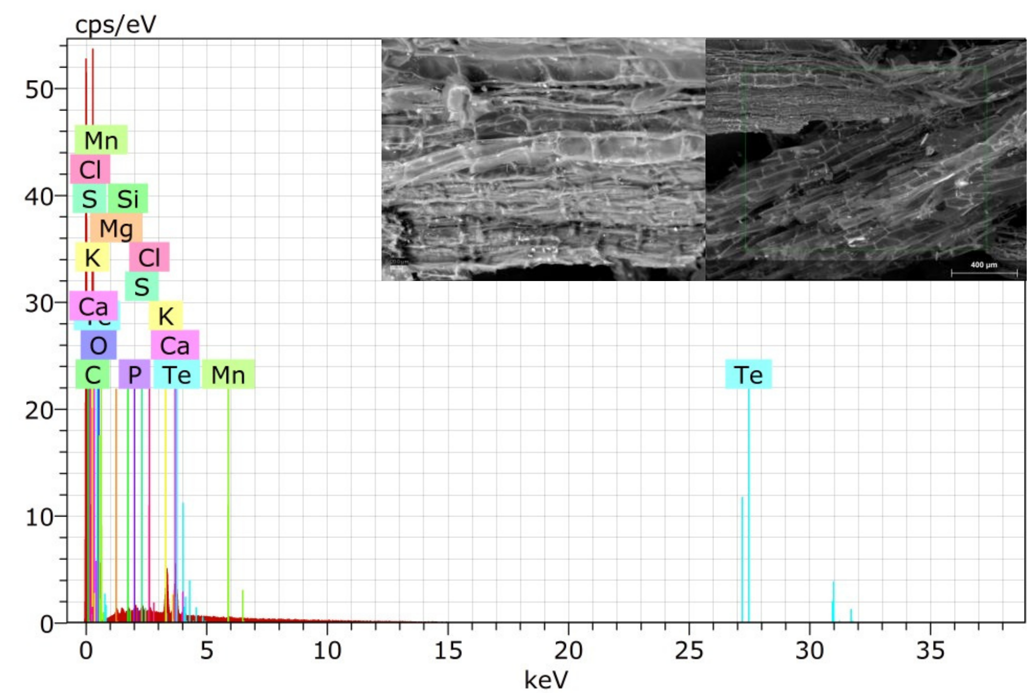
Micrographs of dry water lily (leaves and stem) from Figure 6 indicate that dry biomass is characterized by having a clear, striated, hazy texture and hollow structure that can provide a large surface area for the adsorption of materials (Premalatha et al., 2028).

Figure 6 - SEM micrographs of water lily (*Pontederia crassipes*): a) leaf; b) stem.
Figura 6 – Micrografias de SEM de lírio d'água (*Pontederia crassipes*): a) folha; b) caule



In contrast, in the SEM micrograph of treated *Pontederia crassipes* (Figure 7) some folds in the interconnected structures and roughness in the material are observed. This may suggest that there are sufficient spaces and channels necessary for the adsorption and aqueous transfer of ions (Martinez-Vasquez et al., 2020).

Figure 7 - SEM micrographs and elemental chemical analysis of treated water lily (*Pontederia crassipes*)
Figura 7 – Micrografias de SEM e análise química elementar de lírio d'água (*Pontederia crassipes*) tratado



Elemental chemical analysis indicates the presence of C, O, Cl, K and Si as major elements and Ca, Te, Fe, Mg and P to a lesser extent in leaves and stem of water lily at the two collection sites (Table 1 and 2) respectively.

Table 1 – Elemental analysis in leaves of water lily (*Pontederia crassipes*)
Tabela 1 – Análise elemental em folhas de lírio d'água (*Pontederia crassipes*)

Elements	% mass	
	Coatzacoalcos River	**Casa Blanca Lagoon**
C	29.26	49.02
O	51.93	42.19
Cl	4.45	1.89
K	4.26	2.54
Yes	3.38	0.81
Ca	2.29	0.98
Te	2.13	1.1
Fe	1.57	0.11
Mg	0.70	0.21
P	0.01	0.11

Table 2 – Elemental analysis on water lily stem (*Pontederia crassipes*)
Tabela 2 – Análise elemental no caule do lírio d'água (*Pontederia crassipes*)

Elements	% mass	
	Coatzacoalcos River	**Casa Blanca Lagoon**
C	43.93	47.88
O	44.52	42.61
Cl	4.10	2.1
K	2.8	2.83
Yes	0.11	0.93
Ca	2.25	1.04
Te	1.98	1.09
Fe	ND	0.17
Mg	0.14	0.16
P	0.05	0.05

The high C content (74.3 - 76.7 %) found in water lily lignin in the Mayo River in Navojoa, Sonora, Mexico by Espinoza-Acosta et al. (2022), suggests that this material may be an appropriate raw material for the manufacture of carbon fibers. The difference between the percentage of mass found can be attributed to habitat, temperature, nutrients, and the level of site contamination that influence the chemical composition of the growing plant from place to place (Gómez et al., 1984; Komy et al., 2013; Espinoza-Acosta et al., 2022).

Several authors have pointed out that human commercial practices result in significant amounts of solid waste, highlighting the urgent need for the development of mechanisms to promote rational resource usage and waste control (Dey et al., 2023; Walker & Fequet, 2023; Evode et al., 2021). The findings presented suggest the feasibility of utilizing and manufacturing biodegradable products derived from water hyacinth organic matter (*Pontederia crassipes*), which could help address the global demand for plastics and other highly detrimental compounds.

4. Conclusion

The SEM and FTIR results obtained from the dried and treated biomass of water lily (*Pontederia crassipes*) confirm that it is a natural fiber composed mainly of cellulose and lignin. On the other hand, they suggest the heterogeneity of the biomass surface and a variable chemical content with the presence mainly of functional groups that give it important characteristics for the removal of organic and inorganic pollutants in wastewater treatment, in addition to being used in different technologies as a supplier of nutrients for plants and animals, creation of biodegradable materials, generator of biofuels, among many others.

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