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Research on species diversity and assessment of biodiversity indices of vascular plants in different wetland habitats in Cu Lao Dung district, Soc Trang province

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ABSTRACT

The study was carried out on Cu Lao Dung (CLD), a small island located in the lower Mekong River, in Soc Trang province. The objective of the study was to survey the diversity of species composition and biodiversity indexes of vascular plants here. Since then, the environmental quality has been initially assessed through the research plant group. The study was carried out in 2 years (2018-2020) with qualitative research (following the sampling route) and quantitative research (collecting samples in standard plots. The results of species composition diversity obtained 106 species belonging to 88 genera, 49 families of 2 phyla. The ten most diverse plant families here account for 53.77%. The canal habitat was the habitat with the most species. Woody plants were the dominant group in terms of life forms. The plants in the study area were used medicinally with the rate of 91.51%. Biodiversity index results showed that *Sonneratia caseolaris* was a uniformly distributed species and was also the dominant species in CLD mangroves. Mangrove forests in CLD were tropical plantations and were stable. *Nypafruticans* was the predominant species in lagoon habitats and inundated areas inside and outside the dyke. The groups of plants in this habitat have been greatly affected by relatively stable environmental conditions. The habitats of rivers, canals and lowland gardens were the habitats with the most species. The biodiversity index H reached 5.48, which was an adaptive environment for aquatic plants in the study area.

Key words: Biodiversity index, Cu Lao Dung, Vascular plants, Wetland habitats, Species diversity

Introduction

Cu Lao Dung (CLD) was located in downstream of the Hau River, close to the East Sea, between the two main estuaries of Tran De and Dinh An. CLD has a flat terrain, surrounded by water and dykes and it has typical characteristics of the Mekong Delta with three main natural ecosystems of fresh water, brackish water and saline water (Soc trang province, 2019). These ecosystems have created biodiversity, especially plant species belonging to submerged ecosystems. With the current high rate of saline intrusion, the water quality of the interlaced river system in CLD is affected, the saline water area in CLD is increasing (Can Tho university, 2012; An *et al.*, 2014; Soc trang province, 2019; Department of Natural Resources and Environment of Soc Trang province, 2020). According to studies in the Mekong Delta, the salinity intrusion depends on the sluices, the size of the river, the circulation of the flow and especially the groups of aquatic plants that filter the water (Le *et al.*, 2016; Ogston *et al.*, 2017; Bryan *et al.*, 2017; Soc trang province, 2019; Besset *et al.*, 2019; Rentschler*et al.*, 2020). In wetlands, plants represented a small fraction of the total plant population in the world, but play an important role in ecosystems, as producers, water purifiers, shore keepers, and habitats of many other aquatic organisms (Chambers *et al.*, 2008; Anthony *et al.*, 2015; Chemeris *et al.*, 2019). This flora is a group of organisms that are vulnerable to environmental influences, especially water pollution or saline intrusion, so they are considered environmental indicators (Pond Action, 2002).

In recent years, climate change along with the process of urban and agricultural development that have greatly affected the ecosystem of the Mekong Delta, including CLD (Anthony *et al.*, 2015; Tran, 2019; Soc trang province, 2019). The study of species diversity and number of vascular plant species in flooded areas in CLD is not only meaningful in terms of biodiversity but also useful in assessing environmental impacts here.

Materials and Methods

Study area

CLDis the largest island of Hau River, located in the lower part of Hau River, adjacent to the East Sea, completely separated from the mainland, four sides surrounded by water. The total natural land area of the district is over 26,481.9 ha (Latitude 9°37'39"N and longitude 106°11′8"E). This is also an area with a particularly important position, the term of two large estuaries Dinh An and Tran De. The island has 17 km of coastline. The project area is quite flat but divided into many islands and unstable. Elevation compared to sea level from $+ 0.5m \div + 1.3m$, in which the great part has the height from + $0.7m \div +$ 0,9m. Most of the land area is lower than the high tide, so when the tide is high, water spills into and destroys agricultural production and infrastructure of the population. CLD has a busy river system with more than 360 rivers and canals. Most of the rivers and streams in CLD district are affected by irregular semi-tidal regime, the water level elevation of the two tidal peaks and the two tide legs are not equal. The highest tidal peak is 160 cm (October and November), the lowest is 123 cm (in May and August), the highest tide is -24 cm (November), the lowest is -103 cm (June), average tidal range from 194 to 220 cm. CLD has a diversity of biological resources, especially the mangrove forest. The main biological resources are the agricultural ecosystems in 3 distinct ecological regions, the island top of fresh water, the middle area of brackish water and the end of salty water (Soc trang province, 2019).

Methods

Field investigation method

The study area is divided into different wetland habitats. Each habitat will be applied with compatible field survey methods. For mangroves, selecting random sampling points and locating by GPS, from the edge of the sea and along the cross-section to enter the forest (JOFCA, 1999; Ellison et al., 2012); Although the topography of CLD is relatively flat, it is surrounded by water and separated by dikes to form some low-lying areas, flooded areas, ponds, ditches, low gardens and other areas inside and outside the dyke. In aquaculture areas, field survey method is applied as for a small wetland (Hails, 1997; Ellison *et al.*, 2012); CLD has about 360 large and small rivers stretching from the headto the tail of the dune. The habitats of riversides change continuously. There are 2 typical representative habitats: agricultural and urban habitats. All big rivers were investigated in the field from the dike entering the dune bed to the small tributaries, the sites selected based on the presence of dams and the salinity in the river (Gurnell *et al.*, 2014).

Qualitative (linear survey) and quantitative (standard plot survey) methods for surveying aquatic vascular plants were used in this study (Madsen and Wersal, 2017).

18 routes were surveyed, including 5 routes along the dike from the head to the tail of the island; 2 routes along the main provincial road; 1 route along Con river; 1 route along the small Vam Ho Rach; 1 route along Rach Trang; 2 routes along Con Coc river; 1 route from Rach Sau river to Rach Gia; 1 route from Rach Gia to Rach Binh Linh; 4 routes along irrigation canals and canals of An Thanh 1, Dai An and An Thanh 3.

30 standard plots were selected to survey the diversity of species and calculate the biodiversity index (Fig. 1). Depending on the types of water surface habitats, the standard plots are measured with different sizes, the largest plot is 25mx25m for mangrove habitats, inundated areas inside and outside

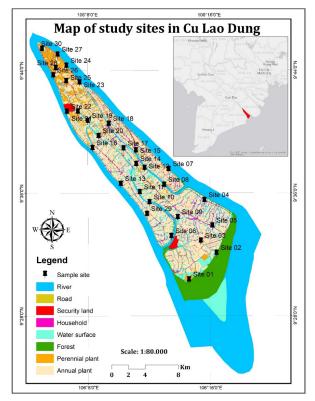
the dyke; plots with dimensions of 10mx10m for habitats of large rivers, canals, ponds and ditches, flooded lowland gardens; The smallest plot is 5mx5m in size for the habitat of the aquaculture area. The standard plots on large rivers, canals and ponds are all taken as the edge of the standard plot and measured out to the riverbed, canals and ponds.

Sampling method

Based on the definition of Diop (2010), all vascular plantsgrow entirely in water; have leaves floating in the water with roots clinging to the ground; the plants float completely on the surface of the water with its roots not clung to the ground; plants growing along the shore with roots clinging to the base of the trunk rising above the water surface; groups of plants living in the intertidal zone and groups of marsh plants were sampled for identification. Samples were collected in 2 seasons (rainy and sunny) for 2 years (2018 to 2020) so that samples with full taxonomic characteristics of aquatic plants could be obtained (Parsons, 2001).

Identification method

Sample collection and identification steps according





to Hassoon*et al.* (2017) and Bowles (2004). Determining the scientific name according to "An illustrated flora of Vietnam" of Ho (1999) and "Flora of Vietnam" (Ban, 2000). Editting family names, genera and species names according to "Checklist of Plant Species of Vietnam" (Ban, 2017) and looking up synonyms at website theplantlist.org (The Plant List, 2003).

Evaluations of species composition diversity, taxonomic levels were made according to the method of Thin (2008), Le (1999), Bertrand *et al* (2006) and Magurran (2004). Statistics on the number of species, genera and families by phylum to calculate the percentage of taxa, the percentage of the 10 richest families of aquatic flora in CLD.

Life forms were determined according to Raunkiaer's division scale with adjustments for aquatic and coastal plants (Ellenberg, 2015). Life forms are divided into upper bud, semi-hidden bud, soil bud, mud bud, and water bud (Schneider *et al.*, 2018).

The values of aquatic plants are assessed based on the benefits of biodiversity including direct and indirect-use values or non-use values (Vermeulen and Koziell, 2007). Conservation values were assessed according to the IUCN red list. The direct-use of species is mainly based on searching documents, groups of uses including medicinal herbs, timber; food; firewood and for other purposes.

Quantitative assessment of biodiversity through indicators such as frequency (F); The abundance (A) and the A/F ratio were calculated according to the following formulas:

Frequency $(F)(\%) = \frac{\text{Number of sample plots with species occurring}}{x \ 100}$
Total number of study sample plots
Abundance $(A)(\%) = \frac{\text{Total number of individuals appearing on all study sample plots}}{2}$
Number of sample plots where the studied species appear

The Shannon-Wiener Diversity Index, H, (Shannon and Wiener, 1963)

$$H' = -\sum_{i=1}^{n} \left(\frac{N_i}{N} x \ln \frac{N_i}{N}\right)$$

Where: H': The Shannon-Wiener Diversity Index n: Number of species N_i : The number of individuals of species i. N: Total number of individuals of all species in the field

Results and Discussions

Species diversity

From the statistical results of plant groups on flooded habitats in CLD, obtaining 106 species be-

longing to 88 genera, 49 families of 2 phyla. The results show that diversity of species composition here, in which, the ratio of Pteridophyta and Magnoliophytawas 2.83% and 97.17%, respectively. The data were shown in Table 1. In the Magnoliophyta, the proportion between the Magnoliopsida and Liliopsida (M/L) is 1.45. The M/L ratio at genus level is 1.69 and at family level 1.76. According to Bognounouet (Bognounou and Ayangma, 2011) the M/L ratio can be used to predict the structure of different forest types (habitats). The flora of Vietnam has an M/L ratio usually 3.2 (Le, 1999). Thus, based on the M/L data, it shows that in the aquatic flora in CLD, dicotyledonous plants still dominate (in terms of total existing species) but with a low rate compared to other habitats in the tropical area (M/L ratio is 3 to 5). This also means that monocotyledonous medicinal plants are well adapted to the flooded habitats of CLD.

According to Granville (1991), the distribution of the number of species (genus, family) according to taxon reflects the specific structure for each flora (Granville, 1991). In which, the percentage of the 10 most species-rich families are considered a reliable evaluation criterion and it shows the flora appearance of the study area. According to the statistics of Le (1999), the 10 richest families of flora in Vietnam, in descending order, include the Fabaceae, Orchidaceae, and Poaceae. Rubiaceae, Euphorbiaceae, Cyperaceae, Asteraceae, Lauraceae, Fagaceae, and the Acanthaceae.

From Table 2, it can be seen that the ten most diverse families in the aquatic flora of CLD belong to the Magnoliophyta. Besides the common plant families of Vietnam, the dominant plant families can also include the Araceae, Convolvulaceae, Moraceae and Verbenaceae. In the flora of Vietnam, the Araceae, Convolvulaceae, Moraceae families are not too diverse in species composition (with from 100 to 150 species) compared to the most diverse families like Fabaceae, Poaceae or Asteraceae

(with from 450 species to thousands of species) (Ho, 1999), but most of the species in the family have many adaptations to aquatic environments, thus becoming the dominant families in the aquatic flora at CLD. The Araceaefamily includes typical species for aquatic organisms such as *Acorus verus*, *Aglaodorumgriffithii*, *Colocasia esculenta*, *Cryptocoryne ciliate*, *Lasia spinosa* and *Pistiastratoides*. Especially, the mangrove species are mostly *Avicennia marina*, *Premnaserratifolia*, and *Clerodendrumserratum*. The Convolvulaceae family with species that tolerate fresh habitats and are mostly vines, creeping along the shore (with shoots sticking to the mud), *Operculinaturpethum*, *Aniseiamartinicensis*, *Ipomoea aquatica*, and *Ipomoea pes-caprae* subsp. *brasiliense*.

According to world statistics, the 10 richest families usually account for 40% to 50% of the total species, and the richest families do not exceed 10% (Le, 1999). Plant statistics in the CLD wetland show that the 10 most diverse families with 57 species, accounting for 53.77% of the surveyed species, and the richest family at 10.38% (the Poaceae family is the most diverse with 11 species) (Table 2). This shows that the flora belongs to a humid tropical structure and is very diverse. However, beside the 10 richest families with a high number of species, the remaining families are monotypic or two species, this structure shows the vulnerability of aquatic flora in CLD.If environmental conditions change, the loss of a single species in the monotypic group can change the structure of the whole flora here (Duraiappah and Naeem, 2011).

With many characteristics of adaptation to adverse conditions such as high tolerance, good dispersal and germination in aquatic environments, genera include herbaceous species such as the genus *Cyperus* (6 species accounting for 5.66%), the genus Digitaria (3 species accounting for 2.83%) is among the 10 most species-rich genera in the study area (Larridon *et al.*, 2011; Jenks, 2015). Notably, the woody group of the genus Ficus also accounted for

Table 1. Diversity of aquatic and coastal flora taxa on CLD, Soc Trang province

Taxon	Family		Genus		Species	
	Number	(%)	Number	(%)	Number	(%)
Polypodiophyta	2	4,08	2	2,27	3	2,83
Magnoliophyta	47	95,92	86	97,73	103	97,17
Magnoliopsida	30	61,22	54	61,36	61	57,55
Liliopsida	17	34,69	32	36,36	42	39,62
Total	49	100	88	100	106	100

No.	Family	Genus	enus	Species	cies
		No.	(%)	No.	(%)
1	Poaceae	8	9.09	11	10,38
2	Asteraceae	8	9.09	8	7,55
3	Cyperaceae	2	2.27	7	6,60
ł	Araceae	6	6.82	6	5,66
5	Euphorbiaceae	5	5.68	5	4,72
5	Fabaceae	5	5.68	5	4,72
7	Convolvulaceae	3	3.41	4	3,77
3	Mimosaceae	4	4.55	4	3,77
)	Moraceae	1	1.14	4	3,77
0	Verbenaceae	3	3.41	3	2,83
	Ten most diverse families (20.41%)				
	Total	45	51.14	57	53,77
		88	100	106	100

Table 2. Ten most diverse families of aquatic and coastal flora of CLD, Soc Trang province

a high proportion (3.77%) with 4 species including *Ficus benjamina* L. var. *nuda, Ficus callophylla, Ficus hispida, Ficus macrocarpa*. All the fruits of these 4 species are food for birds and bats, so they have good seed dispersal ability, and seeds are easy to germinate (Fortune, 1852). Flood tolerance of species of this genus is demonstrated by the root system's ability to hold, resist and take up air (Gamlath *et al.,* 2010). The remaining genera with 77 monotypic genera and 8 genera with 2 species (accounting for 72.64% and 15.09% of total species respectively) Fig 2.

The diversity of the ecosystem leads to a very rich number of plant species in the flooded areas of CLD. Mostly all wetland ecosystems in the Mekong Delta can be found in the CLD from mangroves, to small wetlands and canals (Soc trang province, 2019) (Fig. 3). In this study, the habitats are divided according to structural features, combined with the division of areas with different salinities and different salinities. Habitat groups of CLD can be divided into 3 groups of inundation with different salinity: (1) saline area at the tail of the dune, the location adjacent to the sea including mangroves and estuaries; (2) the brackish water area in the middle of the dune including the low-lying areas inside and outside the dyke, large rivers, small canals and aquaculture areas within the dyke; (3) The freshwater area includes agricultural habitats in the middle of the dune bed and the head of the dune.

Statistics show that freshwater plants still dominate in wetland habitats around residential areas such as small canals, ponds and ditches around houses, small ditches and low gardens. The number of species in pure saline areas such as mangroves or aquaculture areas is less. Saline water is not an optimal condition for plants, so the number of species adapted to saline conditions is always lower than the number of freshwater species (Duraiappah and Naeem, 2011). On the other hand, the number of species in agricultural habitats such as small ditches, gardens, ponds and canals around houses, and small canals are mostly planted trees, which may be the reason for the increase in the number of aquatic plant species in this area. CLD.

The diversity of habitats in CLD also leaded to the diversity of life forms of the studied plant species. The results on the life forms of the species obtained showed a full range of life forms from very forms (Cryptophytes) small stem mesophanerophytes (Phanerophytes) Table 3. Especially the mesophanerophytes group (medium and small) predominates. However, most of them are mangrove groups such as Avicennia, Sonneratia and Rhizophora, species of Ficus also contribute to this diversity. Most of the submerged woody plants in CLD have a broad spectrum of adaptation to different salt concentrations, such species as Sonneratiacaseolaris, Thespesia populnea, Dolichandronespathacea can be found in habitats in saline, brackish and freshwater conditions.

Assessment of the value of biodiversity resources includes direct and indirect use values (Vermeulen and Koziell, 2007). With a high amount of biodiversity as in CLD, the flora here carries many beneficial resources of local and of global values. The ecological effects (breakwater, water purification) of mangroves or wetlands have been demon-



Fig. 2. Diagram showing species diversity of plant genera in CLD, Soc Trang province

strated (Nardin et al., 2020; Bryan, 2017; Hails, 1997). In addition, some specific uses have also been reported, the results are presented in Fig. 4 in which medicinal effect is the highest with 91.51%.

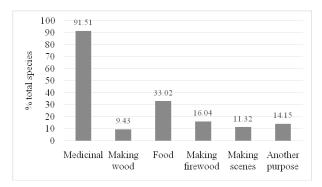


Fig. 4. Graph showing the use values of flora in CLD, Soc Trang province

Evaluation of various indicators

Statistical data in 30 sample plots for the whole wetland habitat in CLD showed 87 species out of 106 species in the flooded areas obtained by linear sampling method. Species that are not included in the quantification plots are those with a small number in the study area, such as *Pandanus tectorius*, Intsiabijura, Syzygiumpolyanthum, Horsfieldiairya; a few are plants such as Morindapersicaefolia, Nymphaea rubra; Most of them are wild species, with little economic value, which have been gradually lost due to agricultural development such as Lygodium japonicum, Glochidion littorale, Aeschynomene indica, Cynodondactylon, Typha angustifolia, Scirpusgrossus; species of unusual habitat in CLD such as sweet acid soil: Melaleuca cajeputi; species are foreign creatures such as Mimosa pigra; Some species can live in both flooded and dry environments but are less likely to S73

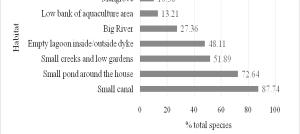


Fig. 3. Diagram showing the habitat of flora in CLD, Soc Trang province

be found in flooded environments such as: Digitariasanguialis, Digitariasetigera, Digitariaheterantha, Paspalum commersonii, Ficus callophylla.

Quantitative results of a number of biodiversity indicators for all wetland habitats at CLD showed that Nypafruticans had the highest frequency of 75%, followed by Ipomoea aquatica and Aglaodorumgriffithii with 50% and 43.3%, respectively, the rest is mostly low frequency. Nypafruticans appeared in 22/30 plots, from saltwater habitats to freshwater habitats, with the highest number of 103 individuals, this is the species with the widest adaptation to a wide range of environmental conditions (Tsuji et al., 2011).

Considering the data of each species, specifically Sonneratiacaseolaris, which is only present in 6/30 plots, is also distributed in saline water, freshwater and brackish water habitats. However, in saline water (mangrove), the density of Sonneratiacaseolaris was the highest with 22 individuals, the remaining

Table 3. Life forms of aquatic and coastal flora of CLD, Soc Trang province

Life form	Number of	%
	species	
Phanerophytes (Ph)	70	66,04
Mesophanerophytes(Me)	20	18.87
Herb phanerophytes (Hp)	16	15.09
Nanophanrophytes(Na)	14	13.21
Lianas phanerophytes(Lp)	13	12.26
Microphanerophytes (Mi)	5	4.72
Cryptophytes (Cr)	36	33,96
Bud clinging mud	11	10.38
Buds in the water	11	10.38
Buds in the ground	8	7.55
Chamaephytes (ch)	4	3.77
Hemicryptophytes (Hm)	2	1.89
Total	106	100

plots in freshwater and brackish water only reached 4 to 6 individuals in 1 plot (Tatongjai *et al.*, 2021). Meanwhile, *Ipomoea aquatica* is also present in 6/30 standard plots, but all these plots only occur in freshwater with very high densities (71 individuals in 1 plot). Thus, it is difficult to apply the A/F ratio for each species to assess the species distribution in the whole study area. When assessing the biodiversity index H for the whole wetland habitat, the data show that using this index for assessment will not be reasonable because the habitats in the study area are too different.

We have divided the study area into 3 main habitat groups including (1) Mangroves; (2) lagoons, ponds and flooded areas inside and outside the dyke; (3) flooded rivers, canals and gardens. Biodiversity indices were assessed including frequency, abundance and biodiversity index H.

In the mangrove habitat, 14 species were recorded, in which, Sonneratiacaseolaris has 100% frequency and highest abundance at 13.5%, Rhizophora apiculate, Avicennia marina, and Aegicerascorniculatum are also species with high A/F ratio ranging from 0.105 to 0.085. Groups with smaller numbers such as Nypafruticans, Aglaodorumgriffithii, Bruguiera sexangular, and Derris trifolia also ranged from 0.07 to 0.045. Only two species with the lowest A/F ratio, namely Thespesia lampas and Excoecariaagallocha, also reached 0.025. Thus, in mangrove habitat, most species have a Contagious distribution (A/F > 0.05), a few species are randomly distributed (A/F in the range of 0.025 - 0.05) (Huy, 2005). This shows the stability of mangrove habitat in CLD, species in this study area do not compete too harshly with each other. The diversity index H in this region is 3.43. This index shows that the mangrove group in CLD is tropical plantation forest (H ranges from 1.16 to 3.40) (Rad et al., 2009). Sonneratiacaseolaris is the most evenly distributed species here, other species have uneven distribution. The dominant species in CLD mangroves is Sonneratiacaseolaris.

Considering the biodiversity indicators in the lagoon habitat and inundated areas inside and outside the dyke, *Nypafruticans* is the dominant species with a frequency of 83.3% and an abundance of 5.8%. There are 46 species found in the standard plots of this habitat. Most are wild species. The A/F ratio ranges from 0.047 to 0.002. Thus, in wetland habitats inside and outside the dike, there are two different distribution patterns. The group with continuous distribution was found in *Thespesia lampas and* *Plucheapteropoda* (with A/F ratio < 0.025). All remaining species in this habitat have a random distribution with A/F ratios in the range of 0.025 - 0.05. There were no species with A/F index > 0.05.

This shows that the environment in these habitats is continuously disturbed. CLD is on the way of economic development, maybe this is the cause of changing environmental conditions. In addition, the fluctuations of climate change, the possibility of saline intrusion are also the causes of the unstable distribution that prevails here (Soc trang province, 2019; Department of Natural Resources and Environment of Soc Trang province, 2020). Regarding the H diversity index, statistics show that the biodiversity index H reached 5.16. It shows that the environment here is stable and the groups of tropical plants in this area have been affected by environmental conditions (Huy, 2005).

In the habitat of rivers, canals and lowland gardens, 73 species were statistically measured in the standard plots. Species such as Nypafruticans, Ipomoea aquatica, Panicum repens, Aglaodorumgriffithii, Acanthus ebracteatus, Acrostichumaureum, *Commelinadiffusa* are species with high frequency (from 72.73% to 40.91%). Most of the remaining species have low frequency, ranging from 18.18% to 4.55%. The vast majority of species in this habitat are herbaceous or hidden shoots, socalculating frequency and density of these speciesare difficult, mostly depends on the water cover in the plot. In this habitat, there are no species with continuous distribution (A/F < 0.025). Most species have A/F values > 0.05. The dyke around the CLD helps to stabilize the water environment in rivers and canals (Hailu, 2017). The biodiversity index H for the whole habitat reached 5.48. From this data, it can be seen that the environment is stable and favorable for the growth of aquatic plants (Huy, 2005).

Conclusion

The survey results on the diversity of plant species composition in the wetland habitats of CLD showed 106 species belonging to 88 genera, 49 families of 2 phyla. In which, dicotyledonous plants predominated and monocotyledons well adapted to flooded habitats. Besides the 10 common families in Vietnam, in the wetland habitats of CLD, there are also families with a high number of species such as Araceae, Convolvulaceae, Moraceae and Verbiaceae families. The 10 most diverse families here ac-

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counted for 53.77%. Adaptive characteristics such as high tolerance, good dispersal and germination in aquatic environments have helped some genera with herbaceous species accounting for a high proportion of species. The canal is the most species habitat. Woody plants are the dominant group in terms of life forms. 91.51% of the plants in the study area are used medicinally.

Biodiversity indicators such as A/F and H ratios have been evaluated, the results show that *Sonneratiacaseolaris* is a uniformly distributed species and is also the dominant species in CLD mangroves. Mangroves in CLD are tropical plantations and are stable. *Nypafruticans* is the dominant species in the lagoon habitat and inundated areas inside and outside the dike. Other plant groups in this habitat are strongly influenced by relatively environmental conditions. The habitats of rivers, canals and lowland gardens are the most diverse habitats recorded, most of which are herbaceous and hidden shoots. The biodiversity index H reached 5.48 this is an adaptive environment for aquatic plants to be collected.

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Conflict of interest

There is no single conflict of interest generated by this study

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