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Characterization of *Thalia geniculata* and *Eichhornia crassipes* macrophytes to improve their phytoremediation performance

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Abstract:
Background*: Phytoremediation is a plant-based technique to reduce, accumulate or break down pollutants in water, soil and air. Screening for macrophytes often takes their extracts into account and is often not based on chemical composition despite studies showing that they reduce various chemical elements, namely heavy metals and nutrients. Although the physical and mechanical characterization of Thalia geniculata has been done and it has been identified as the best lead accumulator in wastewater treatment trials, its metal and nutrient content in these areas of residence remains to be proven. It is in order to compare some elements accumulated by plants of Thalia geniculata and Eichhornia crassipes that this study is being carried out.*
Materials and Methods*: To achieve this goal, plants of Thalia geniculata and Eichhornia crassipes collected respectively from swamps (Togba, Hèvié, Akogbato and Agla) in southern Benin were calcined.*
The analysis methods used are those of French standardization. Cadmium, lead and nutrients were determined respectively by the atomic absorption spectrophotometer HACH DR 2800, HACH DR 800 and the Büchi Auto Kjeldahl k370 still after mineralization.
Results*: From the results obtained, it emerges that all the ashes of the plants of Thalia geniculata and Eichhornia crassipes, are alkaline and have a high content of mineral elements, particularly at the level of the ash of Thalia geniculata which has a high conductivity (4072 µS / cm). Thalia geniculata plants accumulate more lead (36.21 mg / kg of dry matter) and contain a high level of orthophosphates (83,757.31 mg / kg of dry matter) compared to Eichhornia crassipes plants which are rich in total kjeldahl nitrogen (20,874.75 mg / kg of dry matter) and cadmium (9.94 mg / kg of dry matter).*
Conclusion : *Finally, we remark that the two species contain some havy metal and nutrients. They can take off this elements in domestic wastewater.Based on these results, our research will continue on the combination of Thalia geniculata and Eichhornia crassipes in the reduction of nutrients and heavy metals which constitute the pollutant loads of domestic wastewater.*
Key Word*:Thalia geniculata ; Eichhornia crassipes ; Phytoremediation ;Pollutant loads; Domestic Wastewater*

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I. Introduction

Phytoremediation is a technique for treating domestic wastewater (DHW) from macrophytes[1]. Several plants used in this process Iris, Rushes, *Thalia geniculata* (TG) and *Eichhornia crassipes* (JE) have been used to reduce the pollutant loads of this wastewater with varying yields[2]. Although the leaves of *Thalia geniculata*, which is a plant that grows in swamps, have potential that can be used to make molded Pakistani-type plant wrappers for wrapping food balls called <<akassa>>[3] ; and that it has proven its qualities in reducing nutrients[4] and certain heavy metals contained in backwaters[5]; no study has focused on the different elements that compose it in its growth media. The same is true for *Eichhornia crassipes* which plays an important role in the reduction of nutrients, organic loads (COD) and SS at the level of DHW[6,7]. In Côte d'Ivoire for example, the phytochemical screening of extracts from the leaves of *Thalia geniculata*, a plant used in traditional Ivorian medicine in the Krobou regions (Agboville), revealed that it contains large chemical groups (sterols, polyterpenes, polyphenols, flavonoids, catechetical tannins, alkaloids and saponosides) which give it various

therapeutic properties[8]. From the information collected, some macrophytes are able to assimilate heavy metals present in wastewater which are harmful to the environment[9,10]. To determine the content of macrophytes in different elements in order to use them to prepare soap, studies have been based on their ashes[11]. The literature does not provide any information on the elements contained in the macrophytes of *Thalia geniculata* and *Eichhornia crassipes* used in lagooning.
To fill this gap, we looked at the physico-chemical parameters, nutrients and metallic trace elements (Cd and Pb) contained in the ash of *Thalia geniculata* and *Eichhornia crassipes.*

II. Material And Methods

Plants materials : The plants materials consists of macrophytes of *Thalia geniculata* collected in Benin in the districts of Togba, Hèvié and Fidjrossè kpota more precisely in the Akogbato swamp and those of *Eichhornia crassipes* were collected in the 13th district of Cotonou at the level of Agla water collector.

Photo 1: Harvesting of *Thalia geniculata* (a) and *Eichhornia Crassipes* (b) macrophytes

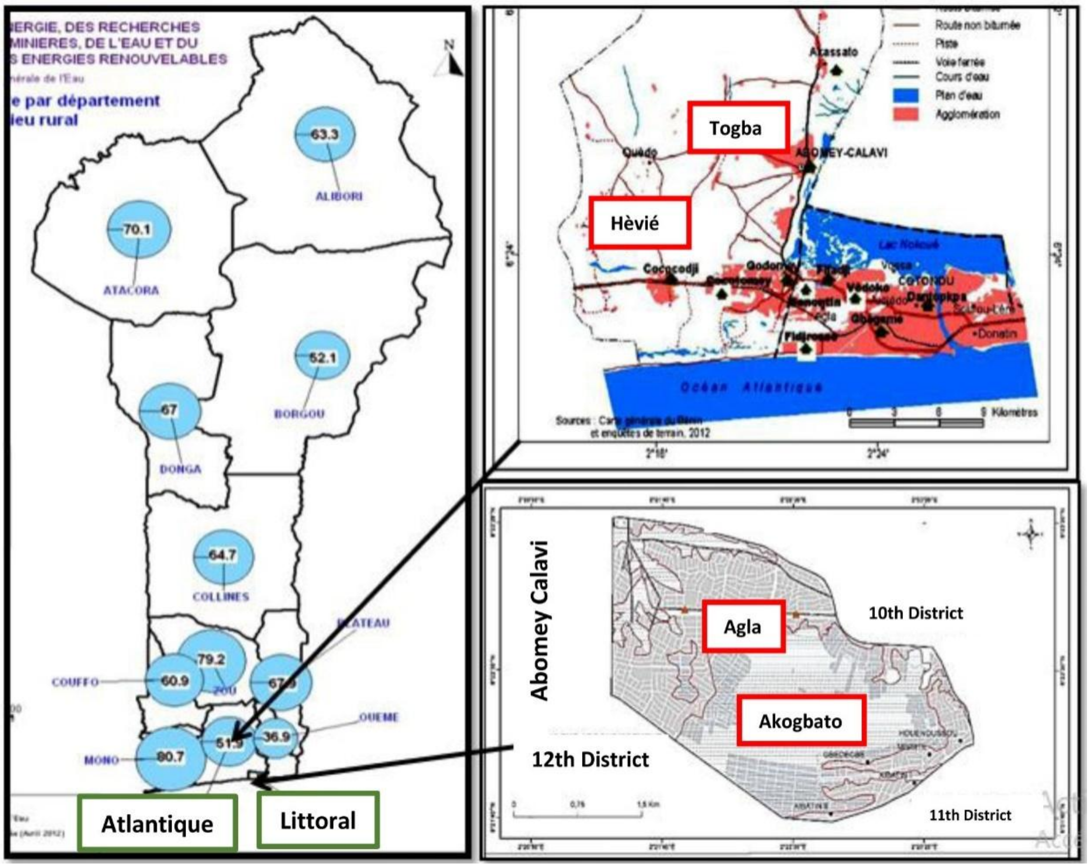

Figure 1: Map of Benin and the town of Cotonou and Abomey-Calavi with macrophyte harvesting areas*[12]

*https://www.bing.com/images/search?view=detailV2&ccid=367%2Fz%2BPl&id

The macrophyte of *Thalia geniculata* is a helophyte of the Marantaceae family. It is a plant with a horizontal and vertical rhizome that grows spontaneously in the natural environment (swamps) and forms mono-specific fields. It is a very productive species that thrives in specific areas and is part of a commercial circuit of local exploitation. Geranylfaresol extracted from the leaves of *Thalia geniculata* is used to control the K_1 strain of plasmodium falciparum[13]. The aqueous decoction of this plant associated with Nauclea latifolia makes it possible to treat malaria in traditional medicine in Benin[14]. The use of *Thalia geniculata* leaves in the diet suggests that it is apotential producer of provitamin A for childreenof 6 to 36 months in Africa[15]. The experiment is carried out in the city of Cotonou characterized by a subequatorial climate with two rainy seasons (April-July, October-November) and two dry seasons (August-September, December-March) marked by an average rainfall of 1200 mm/year . The average temperature varies between 24 ° C and 32 ° C. The macrophytes are stored in mini-basins containing water from the drinking water distribution network of the National Water Company of Benin (SONEB).

Analysis methods Settings

Determination of the water (W) and total organic matter (MOT) content of macrophytes

Freshly harvested and weighed macrophytes (fresh weight: PF) were dried at room temperature for forty-five (45) days to determine dry weight (PS). The water content (TE) of the macrophytes is determined according to the formula below.

$$\mathbf{TE} = \frac{\mathbf{PF - PS}}{\mathbf{PF}} * \mathbf{100}$$

Total Organic Matter (MOT)was determined by the loss on ignition at 600 ° C according to the American standard (Apha, 2005). To do this, the macrophytes placed in a porcelain dish previously dried for 45 days at room temperature were weighed and then placed in a Nabertherm C290 muffle furnace at 600 ° C until constant weight. After cooling in a desiccator, the capsule and the ashes were weighed. The percentages of MOT were determined as follows: Let us call M_o (g) the mass of the empty capsule, M_1 (g) the mass of sample and capsule assembly before calcination and M_2 (g) the mass after calcination.

$$\mathbf{MOT} = \frac{\boldsymbol{M_1 - M_2}}{\boldsymbol{M_1 - M_0}} * \mathbf{100}$$

Determination of the Total Organic Carbon (COT) and Ash content of macrophytes

The Total Organic Carbon (COT) and Ash contents of the macrophytes were determined respectively by the loss on ignition method with the formulas below.

$$\mathbf{Ash = 100 - MOT} \quad \text{et} \quad \mathbf{COT} = \frac{\boldsymbol{MOT}}{\mathbf{1,8}}$$

The Orthophosphates (PO_4^{3-}) and the Total Phosphorus (P-PO_4^{3-}) are determined on the diluted mineralizations by a colorimetric measurement of the phosphomolybdic complex formed using the DR / 2800 molecular absorption spectrophotometer according to the standard (AFNOR, T90-023).

Total Kjeldahl Nitrogen (NTK) is determined using the AFNOR NFT90-110 method. It is a mineralization of organic nitrogen into ammoniacal nitrogen by sulfuric acid. The ammonium obtained is determined by acidimetry after distillation with the Büchi Auto kjeldahl k370 still.

Total Nitrogen (N-NO_3^-) and Nitrates (NO_3^-) are measured according to the colorimetric method by diazotization according to standard NF EN ISO 13395 (1994) after reduction following passage through a cadmium column with a HACH DR / 800 spectrophotometer[16].

Determination of lead and cadmium in macrophytes

The metallic trace elements (lead and cadmium) were determined from samples prepared from macrophytes ash. The ash is dissolved in 2 mL of hydrochloric acid solution of normality 6 N, which is evaporated on a hot plate at 125 ° C. The more or less viscous residue obtained is dissolved again and recovered using 0.1M HNO_3 in a 100 cm^3 flask. The solution thus obtained is used, after dilution or not, for the determinations of lead and cadmium with the atomic absorption spectrophotometer (AAS) HACH DR 2800 according to certains researchers[17].

III. Result

Organic Matter (MO), Total Organic Carbon (COT) and Ash content of *Thalia geniculata* and *Eichhornia crassipes* plants

The characterization of *Thalia geniculata*macropytes, (collected at Akogbato: TGA, at Togba: TGT and at Hevie: TGH) and*Eichhornia crassipes* macrophytes collected at Agla (JE), led to the measurement of the moisture content (W), total solids (TS), organic matter (OM), total organic carbon (COT) and ash. These values are listed in Table no 1.

Table no1: Moisture, total solids, organic matter, total organic carbon and ash content of macrophytes

Macrophytes	W (%)	TS(%)	MO(%)	COT(%)	Ash(%)
TGA	87.45±0.28	12.54±0.67	82.99±0.07	17.00±0.28	46.1±0.1
TGT	75.50±0.37	24.49±0.50	88.24±0.06	11.75±0.37	49.02±0.01
TGH	83.90±0.83	16.09±0.52	89.25±0.14	10.74±0.05	49.58±0.04
JE	80.38±0.45	20.47±0.67	88.58±0.07	9.12±0.72	49.27±0.54

Through this table, we notice that the water content of *Thalia geniculata* macrophytes (TGA: 87.45 ± 0.28%; TGH: 83.9 ± 0.83%) is greater than that of *Eichhornia crassipes* (JE: 80.38 ± 0.45%). The macrophytes have a high water content. Total solids at all macrophytes range from 12.54 ± 0.67% (TGA) to 24.47 ± 0.67% (JE). All macrophytes have the same organic matter content (around 88%) except for the macrophytes of *Thalia geniculata* from Agla which have a low organic matter content (TGA: 82.99 ± 0.07%).

In contrast, TGA contain a high total organic carbon content (17.00 ± 0.28%) compared to the macrophytes of TGT (11.75 ± 0.37%) and TGH (10.74 ± 0.05%). The total organic carbon content of *Eichhornia crassipes* (JE) is low (9.12 ± 0.72%) compared to that of different *Thalia geniculata* plants. All macrophytes have an ash content of less than 50% of the dry matter mass. Akogbato*Thalia geniculata* (TGA) macrophytes have the low ash content (TGA: 46.1 ± 0.1%). To better compare the different macrophytes, figure no 2 below has been drawn.

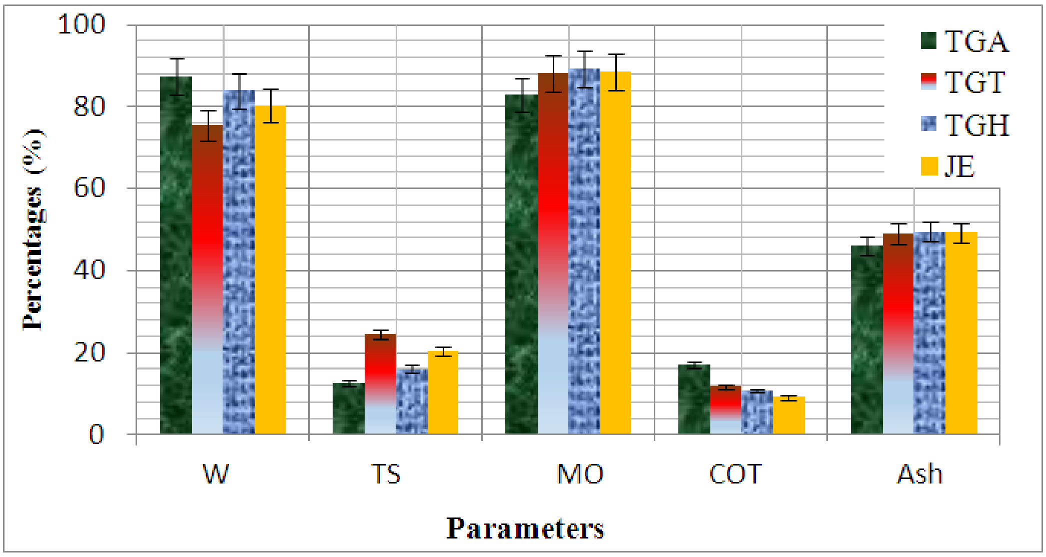

Figure no 2: Physico-chemical parameters of macrophytes

It is noted that the histograms of water content and total organic carbon of macrophytes of *Thalia geniculata* of Agla exceed those of TGH, TGT and JE.The percentages of total solids and ash of *Thalia geniculata*macrophytes of Togba are greater than or equal to those of *Eichhornia crassipes*.

Physicochemical characteristics of the ash of *Thalia geniculata* and *Eichhornia crassipes* plants

The physicochemical parameters of the ash are grouped together in table no 2.

Table no 2: Physicochemical characteristics of the ash

Macrophytes	pH	CE (µS/cm)	TDS (mg/L)	T (°C)
TGA	9.82±0.21	4072.00±3.15	2038.00±25.01	27.10±1.12
TGT	9.30±0.14	2747.00±21.05	1374.00±31.12	2.40±1.58
TGH	8.52±0.08	2220.00±41.57	1111.00±21.01	27.40±1.54
JE	9.92±0.01	879.00±7.12	438.00±3.07	27.20±0.01

The pH varies between 8.52 and 9.92. *Eichhornia crassipes* is slightly more alkaline (pH: 9.92) compared to the ash of macrophytes of *Thalia geniculata*(TGA: 9.82; TGT: 9.30;TGH: 8.52).The values in the table show that all the ashes of the macrophytes studied are alkaline. The conductivity of TGA is very high (4072 µS/cm) and is one and a half to two times higher than that of the ashes of TGH (2220 µS / cm) and TGT (2747 µS / cm). In addition, the ashes of TGA have a high total dissolved solids content (2038 mg / L) compared to those of TGT and TGH which are respectively equal to 1374 and 1111 mg/L.

The experiments are done in same climate condition and the same temperature (27.30°C).

Heavy metal content of macrophytes of *Thalia geniculata* and *Eichhornia crassipes* plants
Cadmium and lead were determined in the macrophyte samples. The values obtained are entered in Table no 3.

Table no 3: Plomb and Cadmium content of macrophytes

Macrophytes	TGA	TGH	TGT	JE
Pb (mg/kg per dry matter)	36,21±0,01	43,25±0,01	40,55±0,01	21,86±0,03
Cd (mg/kg per dry matter)	6,45±0,06	15,57±0,01	7,40±0,01	9,94±0,01

Macrophytes have a metal content between 6.450 and 43.253 mg / kg per dry matter with a high lead and cadmium content for TGH (43.253 mg / kg per dry matter) and 15.570 mg / kg per dry matter. These observations allow us to say that the waters of Hevie swamp content a big value of lead and cadmium than those of Agla (Pb:36.21 mg / kg per dry matter; Cd:6.45 mg / kg per dry matter;) and Togba swamps (Pb:40.55 mg / kg per dry matter; Cd:7.40 mg / kg per dry matter;). JE macrophytes also accumulate a high lead content equal to 21.869 mg / kg per dry matter and a high cadmium content equal to 9.94 mg / kg per dry matter;but concentrate more cadmium than the macrophytes of *Thalia geniculata* which has high concentration of lead. Figure no 3 below shows the histograms of metal accumulation by macrophytes. All macrophytes accumulate cadmium and lead. TGA and TGT accumulate the same amount of cadmium per gram of dry matter.

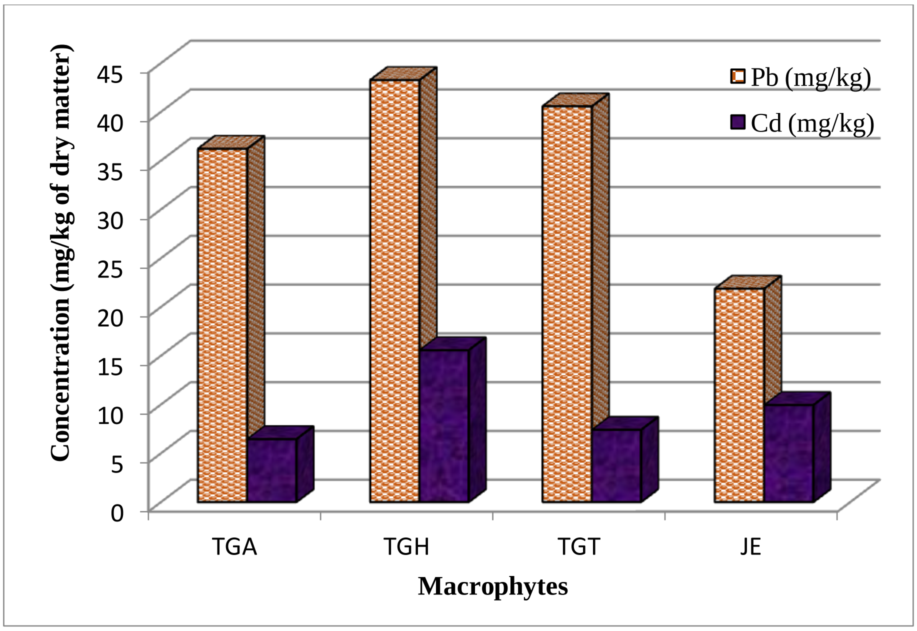

Figure no 3: Amount of Pb and Cd accumulated by macrophytes

Nutrient content of macrophytes of *Thalia geniculata* and *Eichhornia crassipes* plants
The differents values obtained for the nutrients content in the macrophytes are recorded in table no 4.

Macrophytes	PO_4^{3-} (g/kg)	NTK (g/kg)	NO_3^- (g/kg)	P- PO_4^{3-} (g/kg)	N-NO_3^- (g/kg)
TGA	83.76±1.10	17.26±0.01	36.06±0.02	27.37±0.20	81.41±0.01
TGH	10.69±0.06	12.11±0.05	41.03±0.01	34.93±0.06	92.62±0.02
TGT	9.49±0.01	17.18±0.03	54.27±0.15	31.01±0.15	12.25±0.01
JE	18.9±0.01	20.88±0.01	14.88±0.17	5.98±0.04	33.59±0.02

Table no 4: Nutrients contents in macrophytes

There is a strong presence of nutrients in macrophytes TGA which contain a high contration of orthophosphates (83.76 g / kg per dry matter) and total kjeldahl nitrogen (17.26 g / kg per dry matter). TGT plants contain a high nitrate content (54.27 g / kg per dry matter). Likewise, *Eichhornia crassipes* plants are rich in total kjeldahl nitrogen (20.87 g / kg per dry matter) and orthophosphates (18.28 g / kg per dry matter). The less values are obtained for certains macrophytes in the orthophosphate case (TGT:9.49g / kg per dry matter), in total kjeldahl nitrogen (TGH: 12.11 g / kg per dry matter) and nitratous case(JE: 14.88 g / kg per dry matter).The elements levels are shown in figure 4 as a histogram.

The evolution of the different histograms shows that the TGA, TGH and TGT plants absorb kjeldahl nitrogen in the same way with a slight difference in the level of nitrates. On the other hand, they accumulate phosphorus and orthophosphate ions differently.Histograms of *Eichhornia crassipes* (JE) plants show small sizes for orthophosphates, phosphorus and nitrates. We can say that macrophytes of *Thalia geniculata* has a taller histogramm for nitratous and orthophosphates than *Eichhornia crassipes* which has the hight level of total kjeldahl nitrate.

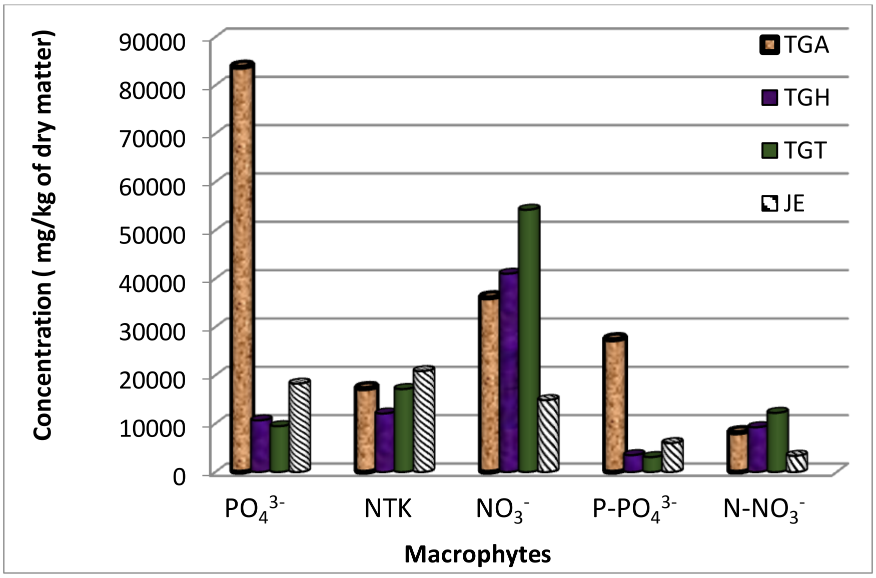

Figure no 4: Nutrients levels in macrophytes

IV. Discussion

The table no 1, show that the different values may be justified by the differences in morphology between the macrophytes of *Thalia geniculata* and *Eichhornia crassipes. Thalia geniculata* macrophytes would be effective in reducing wastewater volumes when used to treat this wastewater in the lagoon. Such a remark has been observed by Jiménez (2017)[18]. These contents are unevenly distributed in the different macrophytes. It can be said that macrophytes absorb minerals and nutrients differently depending on their availability in the medium[19]. The total organic carbon content of *Eichhornia crassipes* (JE) is low (9.12 ± 0.72%) compared to different *Thalia geniculata* plants. This can be justified by the fact that *Thalia geniculata* plants accumulate more organic compounds than *Eichhornia crassipes* plants. Macrophytes of *Thalia geniculata* can reduce organic compounds in soils and wastewater using phytoremediation. These results are one line with those obtained by Justyna[20]. We can say that the level of mineral salts is high in the macrophytes of *Thalia geniculata* of Hevie, Togba and *Eichhornia crassipes* of Agla. Such an same observation was made by another researchers like Bouroka[21]. Nabir was able to relate young macrophytes to their low ash contents when he had set up the methods of sampling citrus leaves in Morocco for foliar diagnosis[22]. From the ash contents, it can be said that TGA macrophytes are younger than TGH. To better compare the different macrophytes, figure no 2 drawn, help us to note that the histograms of water content and total organic carbon of macrophytes of *Thalia geniculata* of Agla exceed those of TGH, TGT and JE. This shows that *Thalia geniculata* macrophytes have a strong capacity to absorb water and accumulate COT in their vegetative systems regardless of their environment of origin[23]. The percentages of total solids and ash of *Thalia geniculata* macrophytes of Togba are greater than or equal to those of *Eichhornia crassipes*(JE). This shows that Togba macrophytes contain more mineral salts than JE. Those of the Hevie and Agla macrophytes are similar to those of *Eichhornia crassipes*[24]. It is believed that *Thalia geniculata* macrophytes due to their high water, total solids, organic matter and ash content, accumulate mineral elements, organic matter and can be used in planted systems to treat domestic wastewater.

The table 2 show that the alkalinity of the ash is certainly indicative of the presence of the ions of the alkali metals and alkaline earth metals[25]. The high conductivity of these ashes reinforces this presumption of the presence of these ions[26]. The nature of the soils, of these last two zones which are almost identical (sandy strip, shorelines, and a plateau of bar land) compared to a soil composed of coarse sand and fine marine greyish silty sand may argue in favor of these results[27]. The value of total dissolved solids can contribute to the alkalinity of the macrophyte ash[28]. *Eichhornia crassipes* is slightly more alkaline (pH: 9.92) compared to the ash of macrophytes of *Thalia geniculata*[29]. These results show that the physico-chemical characteristics of macrophytes depend on their growth zone and the type of macrophytes[30].

The table no 3 show that TGH macrophytes accumulate more Pb than those of TGT and TGA and have a better rate of cadmium accumulation. The activities carried out or the household waste discarded by the populations in these swamps can be a potential source of lead and cadmium[31]. Such an observation was made by Soclo and Sanny[32,33]. These results corroborate those obtained by Brown[34]. JE macrophytes also accumulate a high lead content equal to 21.869 mg / kg per dry matter and a high Cd content equal to 9.94 mg / kg per dry matter but concentrate more cadmium than the macrophytes of *Thalia geniculata* which are rich in lead. These observations show that macrophytes concentrate the metals of their different development media differently[35]. Consequently, they can, by phytoremediation, participate with an interesting effect in the elimination of metals from polluted water.

In figure no 3, through the histograms of metal accumulation by macrophytes, we can see that TGT accumulates three times more. *Thalia geniculata* macrophytes accumulate lead much more significantly than cadmium. TGA and TGT accumulated slightly less than TGH. We can deduce that the Hevie site is more polluted by metals (Cd and Pb) than the other two sites of Agla and Togba. The amount of lead and cadmium accumulated by *Eichhornia crassipes* is significantly less than that of *Thalia geniculata*[35]. The level of lead concentrated by macrophytes greatly exceeds the CODEX STAN standard which limits it to 0.3 mg / kg of dry matter[36]. The same remark is done for cadmium contents in the differents macrophytes (0.05 to 1 mg / kg). This value corroborate those obtained by Chen in 2004[37]. The different metal concentrations in macrophytes confirm that macrophytes can accumulate heavy metals in different parts of their plant organism[38]. *Thalia geniculata* plants accumulated more lead than JE plants. This result can be interpreted in two ways. Either that the swamps of provenance, TG macrophytes are more polluted with lead than cadmium, compared to harvest swamps from JE plants or that TG macrophytes have the capacity to accumulate lead more than plants of *Eichhornia crassipes* which pile up an equally large quantity of cadmium.

From the table no 3, the results reveal that the wastewater from Agla swamps is highly loaded with orthophosphates and that of Togba is loaded with nitrates. Likewise, *Eichhornia crassipes* plants are rich in total kjeldahl nitrogen (20.87 g / kg per dry matter) and orthophosphates (18.28 g / kg per dry matter). These results reveal that different swamps are very rich in nutrients and that macrophytes have different nutrient contents depending on their growth media[39]. The nutrients levels in figure 4 as a histogram show that the organic phosphorus in the TGA plants is higher than TGT and TGH. It means that there is a strong absorption of organic phosphorus in the TGA plants than TGT and TGH. The concentration of total phosphorus obtained in our macrophytes (TGH:34,93 g/kg) are superior of those obtained in the leaves leachates from *Nymphoides cristata* ($P\text{-}PO_4^{3-}$: 15.00 mg/k)[40]. It means that the total phosphorus concentration is less in leaf than the plant. This observation shows that the waters of the Agla swamp are loaded with substances rich in phosphorus. *Thalia geniculata* plants have a high concentration of nutrients. Polomsky made the same point in 2008[41].

The evolution of the different histograms shows that the TGA, TGH and TGT plants absorb kjeldahl nitrogen in the same way with a slight difference in the level of nitrates.

In figure no 4, we observe that *Eichhornia crassipes* macrophytes consumes these nutrients poorly compared to the macrophytes of *Thalia geniculata*. In contrast, the size of the Histogram of total kjeldahl nitrogen (NTK) of macrophytes (JE) is larger compared to those of *Thalia geniculata* macrophytes. However, *Eichhornia crassipes* plants absorb certain nutrients well and confirms the reason why it is used for the removal of nutrients, organic compounds, heavy metals and water pathogens[49]. Plants of *Thalia geniculata* may also play the same role as they take up these nutrients more than plants of *Eichhornia crassipes* macrophytes.

V. Conclusion

Finally, the comparative study based on the characterization of *Thalia geniculata* plants, harvested in three different areas in South Benin (Agla,Hevie, Togba) shows that the plants have the same morphologies with water content and in total organic matter little variable. They absorb water pollutants in the same way and lead to the conclusion that their use in lagooning would effectively contribute to the reduction of gray water pollutants such as *Eichhornia crassipes* (water hyacinth). Thus, further characterization of *Thalia geniculata* and *Eichhornia crassipes* would allow us to see the purification potential compared to other pollutants in order to bring added value to this plant.

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