



## Phytoremediation: Study of the reduction of nutrients in the Sado backwater by *Thalia geniculata* with Python and XLSAT software

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### Abstract

Phytoremediation is an advantageous technique because of the interesting purification efficiency. But it encounters shortcomings related to the stress effect of macrophytes. This research aims to study the purification capacity of *Thalia geniculata* in the reduction of nutrient pollutants in the water body of Sado in Hevie district. For this, the physico-chemical parameters of the water sampled in three zone (zone 1, zone 2, zone 3) of the body of water were determined in accordance with the French standard. There is a reduction in the nutrient loads of the waters from zone 1 to 3. Nitrates and orthophosphates have decreased respectively from 0.97 to 0.76mg/L and from 2.01 to 0.46mg/L. The waters of zone 3 are less turbid (Turb: 15 NTU) and clearer (coul: 193 uca) compared to the others. The principal component analysis of the data with the XLSTAT software showed a strong correlation between suspended solids and orthophosphates on the one hand, then between dissolved oxygen and nitrates on the other. *Thalia geniculata* plants have played an important role in the reduction of nutrients and in the clarification of the waters of the Sado in Hevie. They can be used to treat domestic wastewater. This study will be extended to other macrophytes.

**Keywords:** Phytoremediation, Sado backwater, *Thalia geniculata*, nutrients.

### Introduction

Water supply covers approximately 70% of the Beninese population<sup>1,2</sup>. The remote areas are the most disadvantaged because of the aspect of the ground and the reliefs<sup>1,3</sup>. They don't allow the national water company of Benin (SONEB) to build drinking water supply systems in these areas. The important place that water occupies in human life leads the inhabitants of these areas to frequently use water from rivers, backwaters, swamps, streams (water from the backwater of sado), even domestic wastewater, or water from collectors to satisfy their vital water needs<sup>4,5</sup>. Many authors observed that areas waters are polluted by many pollutants like nitrarouse, orthophosphorus, element metallic traces, organic matter<sup>6</sup>. The backwater like lagoon water are also contaminated by chemicals pollutants and caused many dangers of the local fisheries developpment<sup>7</sup>. Through these behaviors, they are often confronted with waterborne diseases since the quality of these waters leaves something to be desired<sup>8,9</sup>. To overcome its problems in the sub-Saharan countries where the thorny question of water is discussing (Algeria, Tunisia, Morocco etc.), research has revealed that municipal officials base themselves on the means of wastewater recycling such as filtration, lagooning, phytoremediation or water receptacles planted with purifying

macrophytes<sup>10-12</sup>. In Benin and another country, several water bodies are filled with several macrophytes such as *Thalia geniculata*<sup>13,14</sup>. The researches take one the lake help to understand the behavior of some plants<sup>15</sup>. To achieve this goal, this researcher investigate in this field to bring outthe behavior of macrophytes of *Thalia geniculata* on bodies of water of Sado. For this, tests to reduce pollutants in gray water based on purification basins fitted with macrophytes such as plants of *Eichhornia crassipes*, water lettuce, duckweed and *Thalia geniculata* have been carried out and have been observed that these macrophytes eliminate nutrients and certain metals (Pb and Cd)<sup>16-18</sup>. To control the behavior of *Thalia geniculata* on a large scale in order to make use of it in phytoremediation, the study focused on the river of the Sado bridge which during its circulation crosses the beds of *Thalia geniculata*.

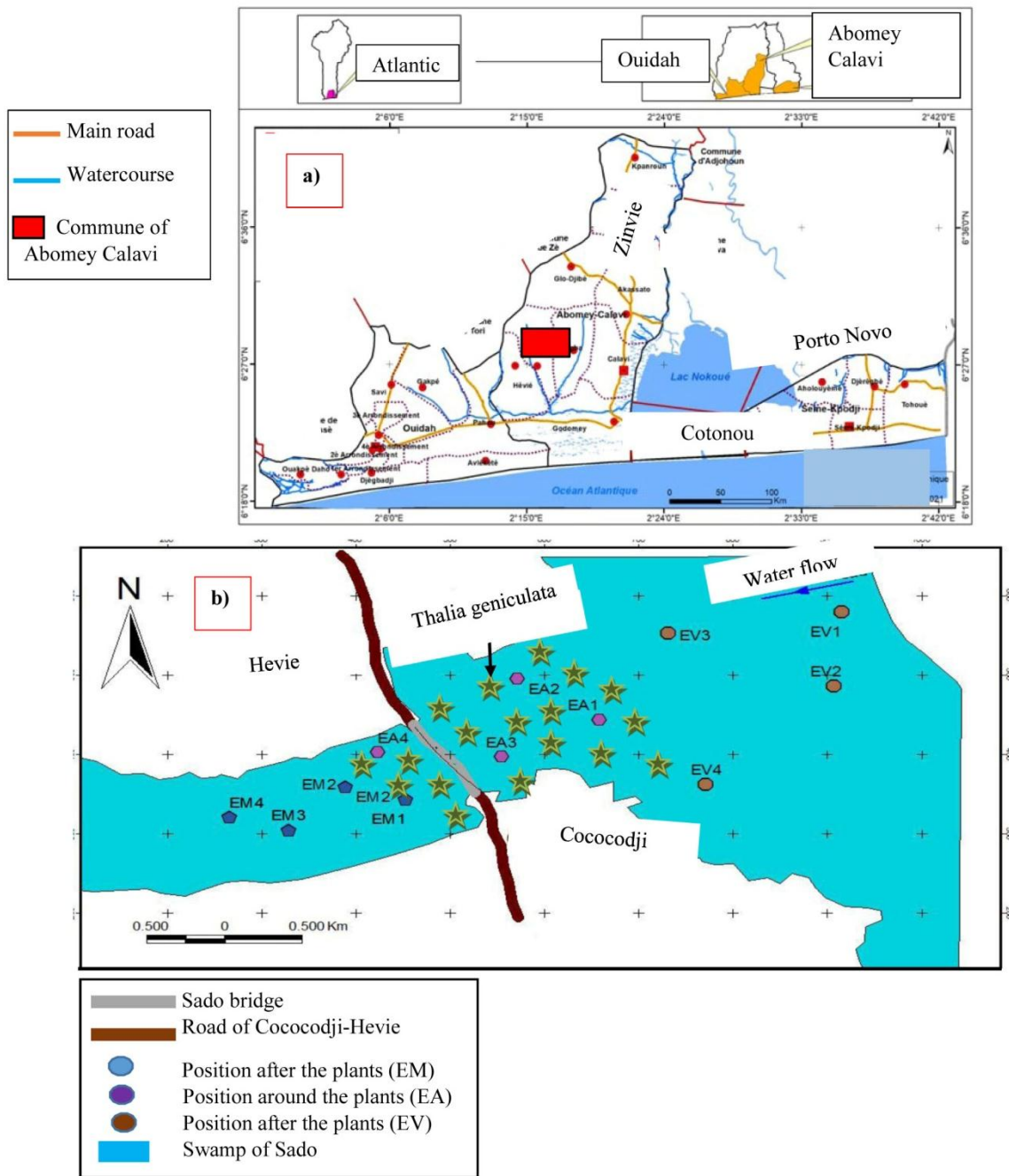
### Materials and methods

**Materials :** The study is conducted in the commune of Abomey Calavi (South Benin) characterized by a subequatorial climate with two rainy seasons (April-July, October-November) and two dry seasons (August-September, December-March). The average rainfall in this town is 1200mm/year and the average temperature varies between 24°C and 32°C. The water samples

were taken in polyethylene bottles with a capacity equal to 1.5L previously washed and rinsed from three areas of the Sado bridge water body (Figure-1)<sup>19</sup>.

The sampling technique was based on that approved by Ifemer<sup>20</sup>. The method of Afriani was also used and modified according to our laboratory conditions<sup>21</sup>. The water samples were taken at a depth equal to 10 cm from twelve (12) positions, such as before the macrophytes *Thalia geniculata* (EV), around the plants (EA) and after the macrophytes (EM). All swamp

water samples that have been taken from each zone were introduced into 1.5L polystyrene bottles and are named according to each zone. The swamp water of Sado is used in part by the good ladies to cultivate the plants of *Thalia geniculata* in order to sell the leaves to traders of corn paste. The aspect of the water of the Sado bridge favored the installation of a gravel sales site near the bridge in order to rid them of dirt. The water samples collected before, after and at the level of the *Thalia geniculata* macrophytes were found at the level.



**Figure-1:** (a) Map of the district of Hevie (South Benin) (b) Backwater of Sado.

**Methods of analysis: Physico-chemical parameters:** The analyzes were carried out at the sampling locations, at the Physical Chemistry-Materials and Molecular Modeling Laboratory (LCP3M), of the University of Abomey Calavi, at the Waste Management, Treatment and Valorization Laboratory (LGTVD) of the University of Lomé and Kaba Laboratory for Research in Chemistry and Applications of the National University of Sciences Technologies Engineering and Mathematics. The data analysis software XLSTAT for Excel was used for principal component analysis (PCA). Some formula are also programming with Python language. The different scripts bases on while loop are used to write this program<sup>22</sup>.

The physico-chemical parameters of the wastewater such as: temperature (T), conductivity ( $\chi$ ), total dissolved solids (TDS) were measured using the multi-parameter Combo Hanna waterproof according to standard NF EN 27888. The pH was measured by the electrochemical method with the Mettler Toledo pH meter fitted with an LE 409 probe (NFT 90-008) and the color (Coul) by the APHA Platinum-Cobalt method. The

turbidity (Turb) was determined by the DR/890 colorimeter and the redox potential (EH) was measured with the WTW pH / EH 340i device according to standard NF EN 27888. Dissolved oxygen (Dissolved O<sub>2</sub>) was determined by the WTW pH/O<sub>2</sub> Innolab 740i device according to standard NF EN 25814.

**Pollution parameters:** The suspended solids (SS) were measured with the HACH DR/890 colorimeter according to the NF EN 872 standard. The orthophosphates (PO<sub>4</sub><sup>3-</sup>) and the nitrates (NO<sub>3</sub><sup>-</sup>) are measured according to the NF EN ISO 10304-1 method with the ion absorption chromatograph. Total phosphorus (TP), total nitrogen (NT) and nitrate nitrogen (N-NO<sub>3</sub><sup>-</sup>) were determined by the spectrophotometric method according to standards NF T90-023 and NF EN ISO 13395.

## Results and discussion

**Physicochemical parameters:** The study of the visual aspect of the waters of the marsh was based on a few physicochemical parameters, namely: pH, T, EH, Cond, Coul, Turb and dissolved Oxygen. The values obtained are recorded in Table-1.

### Python Programm to Calculate the Average of Parameters

```
In [3]: while True:
        parameter=str(input("Enter parameter: "))
        n=int(input("numbers of values n="))
        sam=0
        for i in range(n):
            x=float(input(f"x of {i}:" ))
            sam+=x
            average=sam/n
        print(f"average of {parameter} is {average} mg/L")
        F=input("Do you want to continue? yes (y) no (N):", )
        if F!="y":
            break
        print("end")

Enter parameter: Temperature
numbers of values n=4
x of 0:22.5
x of 1:22.3
x of 2:22.6
x of 3:22.6
average of Temperature is 22.5 mg/L
Do you want to continue? yes (y) no (N):y
```

Figure-2: Python programm to calculate parameters averages.

**Table-1:** Physicochemical parameters of the waters of the Sado backwater.

| Zones | T (°C) | Turb (NTU) | Coul (uca) | EH (mV) | Dissolved Oxygen (mg O <sub>2</sub> /L) | Cond (µS/cm) | pH   |
|-------|--------|------------|------------|---------|---|--------------|------|
| EV1   | 22,5   | 86         | 1020       | 87,5    | 4,60                                    | 151          | 5,57 |
| EV2   | 22,3   | 80         | 945        | 85,6    | 4,96                                    | 132          | 5,52 |
| EV3   | 22,6   | 69         | 750        | 73      | 5,01                                    | 97           | 5,30 |
| EV4   | 22,6   | 57         | 685        | 70,3    | 4,95                                    | 104          | 5,45 |
| E1*   | 22,5   | 73         | 850        | 79,1    | 4,88                                    | 121          | 5,46 |
| EA1   | 23,6   | 42         | 402        | 80,0    | 4,91                                    | 117          | 5,61 |
| EA2   | 23,8   | 34         | 395        | 71,5    | 4,60                                    | 109          | 5,40 |
| EA3   | 24,0   | 21         | 388        | 70,1    | 4,65                                    | 98           | 5,36 |
| EA4   | 24,2   | 19         | 347        | 68,4    | 4,64                                    | 92           | 5,43 |
| E2 *  | 23,9   | 29         | 383        | 72,5    | 4,70                                    | 104          | 5,45 |
| EM1   | 24,7   | 27         | 295        | 74      | 4,68                                    | 102          | 5,35 |
| EM2   | 24,9   | 13         | 186        | 68      | 4,44                                    | 98,1         | 5,27 |
| EM3   | 25,1   | 11         | 147        | 76      | 4,55                                    | 65,2         | 5,42 |
| EM4   | 25,3   | 9          | 124        | 74      | 4,69                                    | 35,5         | 5,40 |
| E3*   | 25     | 15         | 193        | 73      | 4,59                                    | 75,2         | 5,36 |

\*Average values.

The water in the swamp has several aspects from the values in the Table-1. Average temperatures range from 22.6 to 25°C. These values are close and vary slightly from zone 1 to zone 3. This shows that the water temperature of the Sado bridge swamp does not depend on the aquatic flora in the environment. Turbidity is high in zone 1 (73 NTU) showing that it is loaded and cloudy. This finding may be due to human activities<sup>23,24</sup>. From zone 2 to zone 3, the turbidity decreased from 29 to 15 NTU. This value is close to that found in the traditional wells of Magoumi<sup>25</sup>. The same observation was done with the turbidity in spring and summer of Baoan Lake which ranged within 15 NTU<sup>26</sup>. We note that the plants of *Thalia geniculata* have acted on the turbidity of the water current which circulates from zone 1 to zone 3. The same observation is made at the level of the color of the water which passes from 850 to 193 uca when leaving zone 1 for zone 3. The color value of the water downstream (193 uca) is lower than those of the water after the macrophytes (850 uca) and confirms the clear appearance of this water<sup>27</sup>. Dissolved oxygen varies little around the value of 4.70 mg O<sub>2</sub>/L. Such a remark is made on the waters of Lake Grand Brage in Ouagadougou<sup>28</sup>. These values show that the plants of

*Thalia geniculata* do not have a great effect on the dissolved oxygen since the Sado backwater is in perpetual contact with the oxygen in the air and that its variation is often influenced by the temperature of the environment<sup>29</sup>. The redox potential measurements are high and are respectively equal to 79.1; 72.5 and 73 mV from zone 1 to zone 3. This is justified by the fact that the water is more oxygenated downstream than upstream and that this oxygen does not enter the depth of the water because its high turbidity due to the matter that the zone 1 contained<sup>30,31</sup>. The water conductivity dropped from 121 to 75.2 µS/cm. It appears that the charged particles of zone 1 were stopped by the plants of *Thalia geniculata*. The pH of the waters of the three zones is substantially equal to 5.4, thus showing that the water of the Sado backwater is slightly acidic. This may be due to macrophytes which would have played an important role in the reduction of water anions<sup>32,33</sup>. Figure-3 gives the variation of the parameters of the different areas of the Sado backwater.

From this figure, it can be seen that the three parameters drop as one moves away from zone 1. The turbidity and the conductivity vary slightly at the level of the three zones but the

pace of the conductivity is faster in zone 3. This can be explained by the fact that the macrophytes have absorbed the charged particles at the level of zone 2. This can lead to the settling of suspended solids in zone 3 which present clear water<sup>34,35</sup>. The color curve decreases rapidly in all three areas. This fall is respectively equal to 32.84%; 13.68% and 57.96% in

zones 1,2 and 3. This high yield in zone 3 would justify the clarity of the marsh water but is low compared to that of the literature<sup>35,36</sup>. Turbidity, color and conductivity decrease slowly in zone 2 which is the water purification zone. The general behavior of these physico-chemical parameters on the body of water can be seen in Figure-4.

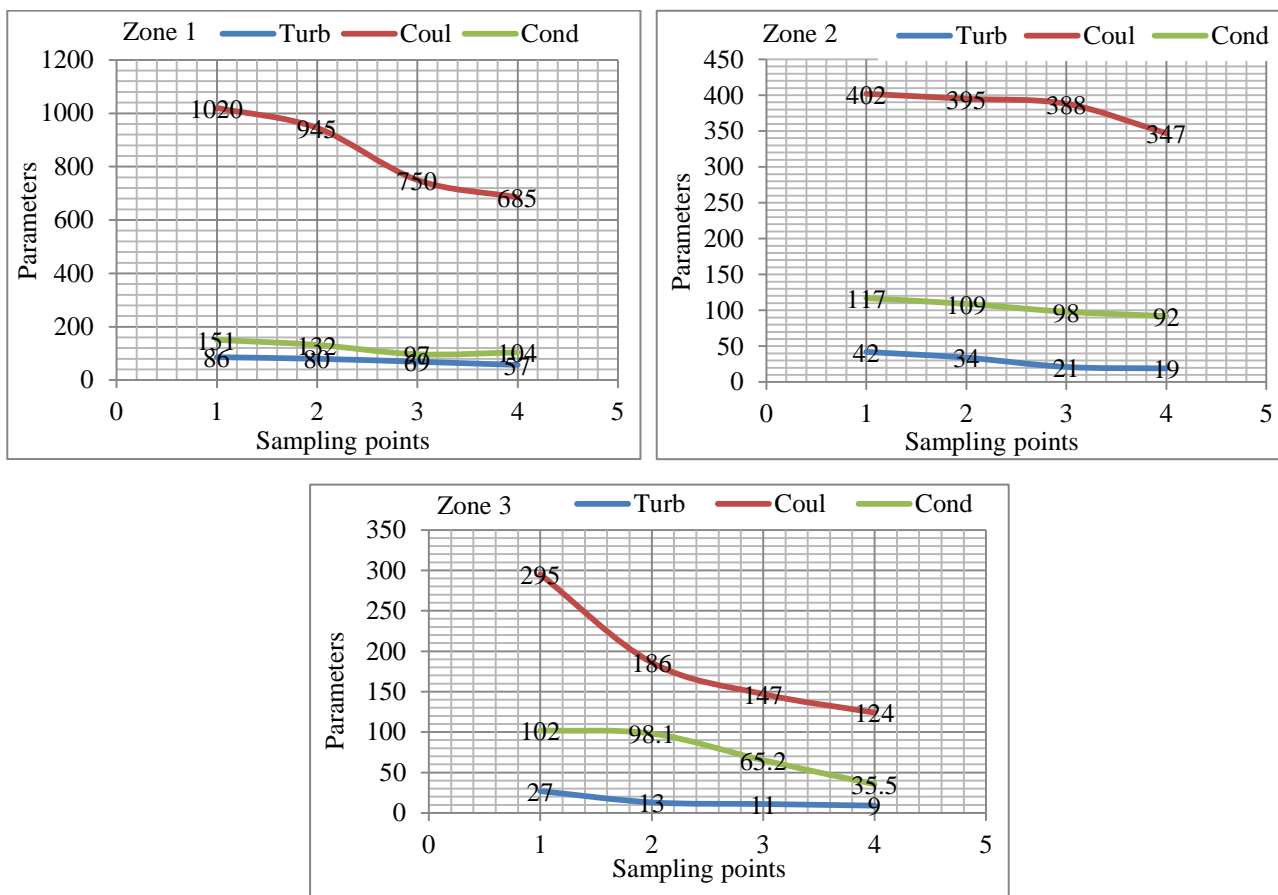


Figure-3: Evolution of turbidity, color and conductivity of marsh waters.

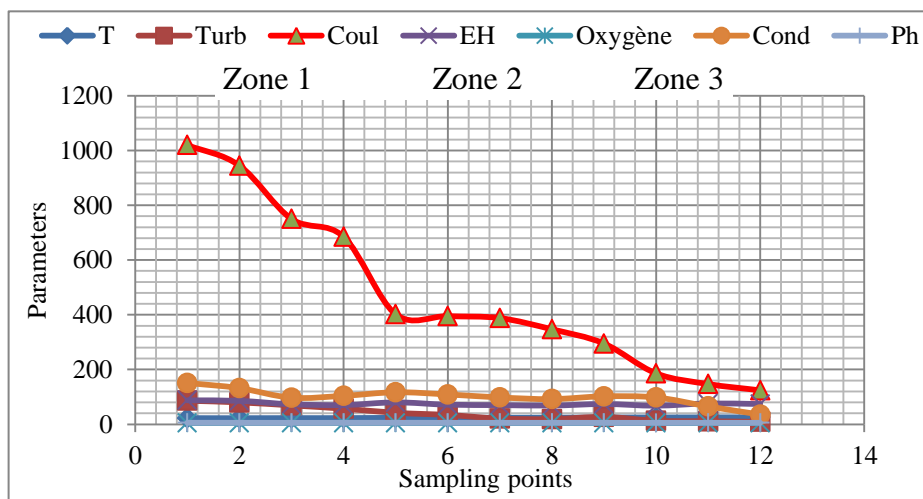


Figure-5: Evolution of some physicochemical parameters of the waters of Sado backwater.

The analysis of this figure shows that the temperature, the pH, the dissolved oxygen content and the redox potential of the waters body of the Sado backwater vary uniformly at the level of the three zones of the swamp. We can say that the plants of *Thalia geniculata* don't act on these parameters at the water level but make the environment slightly acidic because the pH is less than 7 throughout the study area<sup>38,39</sup>. The same observation was done one Mékrou lake which pH was equal to 6.46 and show the acidity aspect of this water<sup>40</sup>. In addition, we notice that the color has dropped considerably from zone 1 to zone 3 while we observe a slow decrease in turbidity and conductivity. It can be noted that there is an interdependence between the physicochemical parameters along the Sado backwater. To better understand the role of *Thalia geniculata* plants in reducing water loads from the Sado backwater, the study focused on the analysis of some pollution parameters.

**Study of some pollution parameters:** Understanding the effect of macrophytes on the waters of the Sado backwater has led to the determination of the content of some pollutants in these waters. The results of these analyzes are recorded in the table below (Table-2).

The analysis of this table shows that MES dropped from 62 to 46mg/L in zone 1, from 24 to 17mg/L in zone 2, and from 15 to 5 mg/L in zone 3. The reduction yield are respectively 25.80%, 29.16% and 66.66%. The macrophytes played a role in the reduction of suspended solids before their passage in zone 3. This favored the settling of small charged particles with the consequence of reducing the color of the water in zone 3. Nutrients (NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>) fell respectively from 1.078 and 2.154 mg/L to 0.890 and 1.809mg/L in zone 1, i.e. by 17.43% and 16.02%; from 0.964 and 0.98 mg/L to 0.756 and 0.759 mg/L in

zone 2 i.e. 21.57% and 22.94% then from 0.921 and 0.511 mg/L to 0.604 and 0.394mg/L in zone 3 i.e. 34.41% and 22.90%. The results show that the nitrate and orthophosphate contents of the water in zone 1 are high. This may be due to the high concentration of total nitrogen (1.190mg/L) and total phosphorus (0.650mg/L) in zone 1. This charge is due to human activities such as agricultural activities, livestock, washing care, garbage<sup>24</sup>. The decrease in these ions in zone 2 is due to *Thalia geniculata* plants which absorb the nutrients for their development develop<sup>10,17,41</sup>. This also explains the low reduction of nitrates and orthophosphates in zone 1 which does not contain macrophytes. Note that the reduction in nitrates did not exceed 50% in zone 2. This finding can be interpreted by the fact that the total nitrogen in the environment undergoes other transformations and then becomes mineralized as nitrate ions were absorbed by macrophytes<sup>42,43</sup>. This is observed through the nitrate and total nitrogen values of the sampling points EV4 (0.890; 1.068mg/L) in zone 1 and EA1 (0.964; 1.043mg/L) in zone 2 then EA4 (0.756; 0.976mg/L) in zone 2 and EM1 (0.921; 0.972mg/L) in zone 3. The rate of nitrate transformed into nitrogen is low but decreases throughout the swamp. It can be said that the phenomenon of denitrification is then slowed down from zone 1 to zone 3. This may be due to the decrease in the micro-organisms responsible for denitrification along the marsh<sup>44,45</sup>. The total phosphorus content is 0.650 mg/L downstream and 0.150mg/L upstream of *Thalia geniculata* plants. This gives an abatement equal to 76.92% for total phosphorus and 21.84% for total nitrogen. At the end of this interpretation, we note that the pollution parameters have decreased along the Sado backwater. The *Thalia geniculata* plants acted on the nutrients of the Sado water body. Figure-5 gives a general overview of the decrease in pollution parameters on the Sado water body.

**Table-2:** Pollutant content of the waters of the Sado backwater.

| Zone | MES | NO <sub>3</sub> -N | NO <sub>3</sub> <sup>-</sup> | N total | PO <sub>4</sub> <sup>3-</sup> | P total |
|------|-----|--------------------|------------------------------|---------|-------------------------------|---------|
| EV1  | 62  | 0,231              | 1,078                        | 1,363   | 2,154                         | 0,715   |
| EV2  | 54  | 0,223              | 0,972                        | 1,205   | 2,097                         | 0,658   |
| EV3  | 50  | 0,217              | 0,940                        | 1,124   | 1,980                         | 0,638   |
| EV4  | 46  | 0,209              | 0,890                        | 1,068   | 1,809                         | 0,589   |
| E1   | 53  | 0,220              | 0,970                        | 1,190   | 2,010                         | 0,650   |
| EA1  | 24  | 0,225              | 0,964                        | 1,043   | 0,985                         | 0,314   |
| EA2  | 21  | 0,201              | 0,881                        | 1,161   | 0,902                         | 0,301   |
| EA3  | 18  | 0,170              | 0,879                        | 1,060   | 0,874                         | 0,298   |
| EA4  | 17  | 0,164              | 0,756                        | 0,976   | 0,759                         | 0,247   |
| E2   | 20  | 0,190              | 0,870                        | 1,060   | 0,880                         | 0,290   |
| EM1  | 15  | 0,201              | 0,921                        | 0,972   | 0,511                         | 0,215   |
| EM2  | 11  | 0,182              | 0,810                        | 0,974   | 0,490                         | 0,175   |
| EM3  | 9   | 0,164              | 0,705                        | 0,920   | 0,445                         | 0,110   |
| EM4  | 5   | 0,133              | 0,604                        | 0,804   | 0,394                         | 0,100   |
| E3   | 10  | 0,170              | 0,760                        | 0,930   | 0,460                         | 0,150   |

It can be seen that the distribution of pollution parameters is not uniform throughout the water body. The suspending matter which was 63.85% in the water of zone 1 before the macrophytes of *Thalia geniculata* was reduced to 24.09% in zone 2 before dropping to 12.04% in zone 3. This decrease may be due to the transformation and arrest of large particles by macrophytes. The nitrogen pollution histograms show the same distributions over the three areas<sup>46,47</sup>. The following levels are noted when moving from zone 1 to zone 3 for nitric nitrogen (37.93%; 32.75%; 29.31%), for nitrates (37.30%; 33.46%; 29.23%) and for total nitrogen (37.42%; 33.33%; 29.24%). The analysis of the results shows that the nitrogen pollution was slightly eliminated on the water body of the Sado backwater. This justifies the statements mentioned above in relation to the

phenomenon of denitrification which could contribute to this low<sup>32,48</sup>. As for phosphate pollution, we note the following distributions (60.00%; 26.26%; 13.73%) for orthophosphates and (59.63%; 26.60%; 13.76%) for phosphorus total. We discover that this pollution has been considerably reduced on the body of water. This corroborates the results of many authors who states that orthophosphates are assimilated by the aquatic flora and the denitrification lead to low reduction of pollutants<sup>49,50</sup>. It is noted that suspended solids and nutrients are eliminated by *Thalia geniculata* plants. But this reduction is low in terms of nitrogen pollution, which is mostly eliminated when macrophytes are used in a domestic wastewater treatment basin<sup>17,51</sup>.

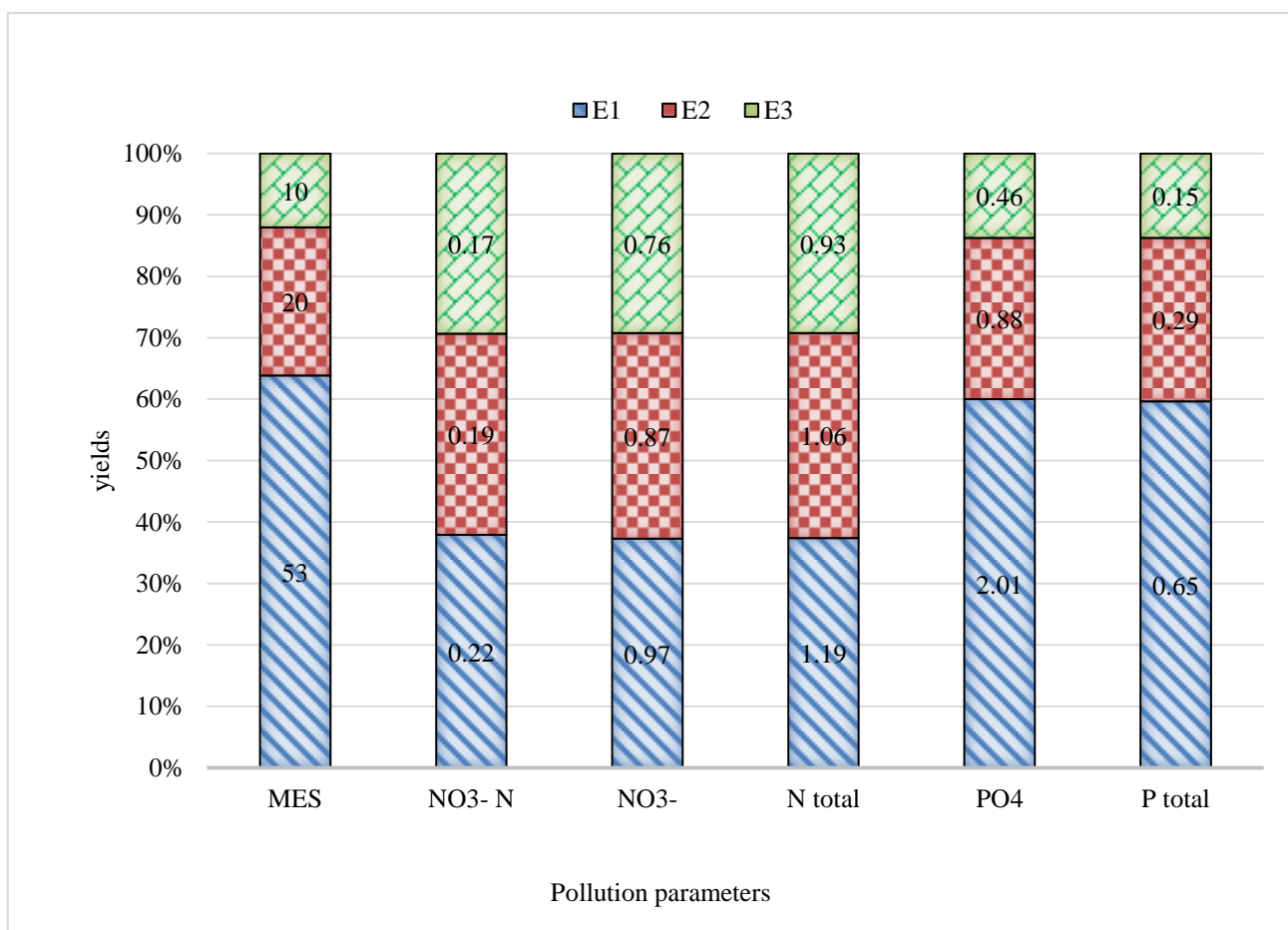


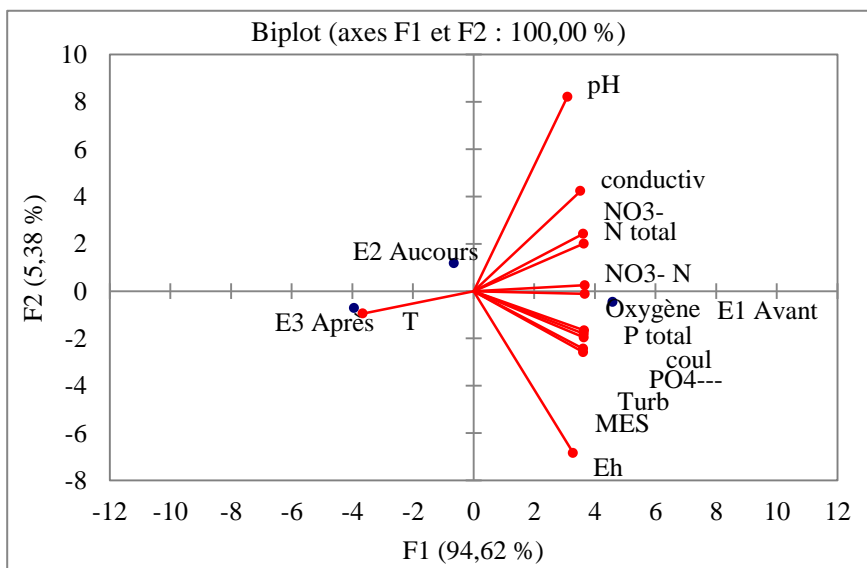
Figure-5: Column diagram of pollution parameters on the Sado backwater.

Table-3: Correlation of physico-chemical parameters with factorial axes.

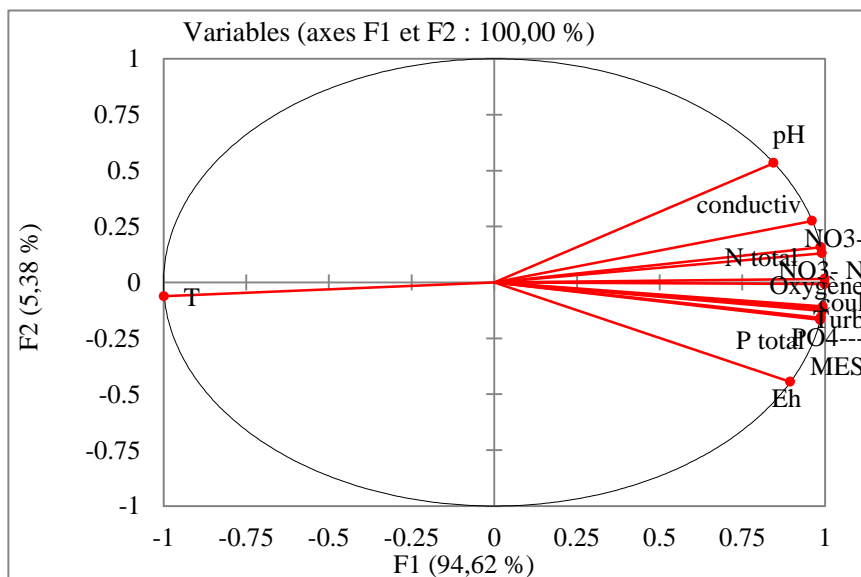
| Parameters | T     | Turb  | MES   | coul  | Eh    | O <sub>2</sub> Dissous | cond | pH   | NO <sub>3</sub> <sup>-</sup> N | NO <sub>3</sub> <sup>-</sup> | N total | PO <sub>4</sub> <sup>3-</sup> | P total |
|------------|-------|-------|-------|-------|-------|------------------------|------|------|--------------------------------|------------------------------|---------|-------------------------------|---------|
| F1         | -0,99 | 0,98  | 0,98  | 0,99  | 0,90  | 1,00                   | 0,96 | 0,85 | 1,00                           | 0,99                         | 0,991   | 0,99                          | 0,99    |
| F2         | -0,06 | -0,15 | -0,17 | -0,11 | -0,44 | -0,01                  | 0,28 | 0,53 | 0,01                           | 0,16                         | 0,130   | -0,13                         | -0,11   |

**Correlation between the different parameters of the areas sampled around *Thalia geniculata* plants:** The results of the principal component analysis on the physico-chemical parameters measured reveal that the first two axes explain 100% of all the variability in the level of the physico-chemical parameters of the sampled water points. This is sufficient for an unbiased interpretation of the results. We will therefore retain these first two axes for the following interpretations. The analysis of the correlation of the physico-chemical parameters with the factorial axes (Table-3 and figure-6) shows that the parameters such as conductivity, turbidity, pH, dissolved oxygen, TDS, suspended solids (SS), color, nitrates, nitric nitrogen, total nitrogen, orthophosphates, total phosphorus are well represented on the first principal component with a good

correlation and contrast the temperature<sup>52</sup>. In other words, the points richest in dissolved oxygen also have a relatively high nitric nitrogen content and very low values of temperature, conductivity, TDS, SS, color and vice versa. The same is seen for color, orthophosphates and TDS one hand and suspended solids and turbidity one other. On the second principal component, pH, conductivity, nitrates, total nitrogen are well represented and contrast orthophosphates, color, TDS, suspended solids, turbidity and redox potential. In other words, the most turbid media are characterized by low values of pH, conductivity, nitrates, total nitrogen and vice versa<sup>52,53</sup>. The projection of the sampled points in the factorial axis plane formed by the two principal components (figure-7) clearly shows the parameters that govern each zone and its position.



**Figure-6:** Correlation of physico-chemical parameters with factorial axes.



**Figure-7:** Projection of the different sampling points in the factorial axis plane.



This Figure shows the first zone (0.924; -0.383) influenced by orthophosphates, color, dissolved oxygen, suspended solids, turbidity and nitrogen pollution. It means that these parameters have very high values in this zone. Zone 2 (-0.130; 0.991) is about 3 measurement units away from zone 1 from right to left and is close to the first axis. This can be explained by the fact that the two zones don't have the same profile and the second one is weakly and only influenced by nitrogen pollution. Temperature has a strong impact on the third zone (-0.793; -0.609) because its profile is governed by temperature. The decrease in pollutants (orthophosphates, color, dissolved oxygen, suspended solids, and turbidity) which act weakly in this area would be responsible for this observation. We note that there are a relationship between orthophosphates and dissolve oxygen<sup>54</sup>. We remark also a difference between water from zone 3 to zone 1 and it is approximately 5 measurement units. According to Pagès, the three zones are remote from the geographical point of view<sup>55</sup>. The same remark was done by Moulia who works one four different types of water<sup>56</sup>.

## Conclusion

This study allowed us to know the degree of pollution of the Sado backwater and the distribution of this pollution along this swamp. The physico-chemical and pollution parameters analyzed decrease when moving from zone 1 located upstream of the *Thalia geniculata* plants to zone 3 which is found downstream of these plants. At the level of zone 2 where the macrophytes are located, the decrease in parameters takes place slowly and this leads to a low yield. Then, the waters of this body of water are clearer at the level of zone 3 after the absorption of nutrient pollutants by the macrophytes.

The principal component analysis centered on the physicochemical and pollution parameters of the three zones made it possible to note geographically that the three zones are distant and the water of zone 1 undergoes the effect of the phosphate and nitrogen pollution which are strongly correlated with SS, turbidity, color and TDS. The water of zone 3 is influenced by temperature and is clear due to the reduction of pollutants by macrophytes in zone 2.

Finally, *Thalia geniculata* plants play an important role in the clarification of surface waters of Sado bridge. In the following work, these plants will be cultivate in swamps water highly charged in order to reduce the pollutants that it contains.

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