



Original Research Article

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## Influence of the Origin of Water Pollution on the Floristic Diversity of Macrophytes of the Mfoundi Lowlands in the City of Yaounde

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### ABSTRACT

The Mfoundi watershed serves as a collector of untreated sewage in the Yaounde urban area. This study on the floristic diversity of this ecosystem was conducted with the objective of determining the influence of water pollution on the distribution of the subservient macrophytes. The inventory based on floristic records from Braun-Blanquet abundance-dominance coefficients was carried out in 90 plots of 5 m x 5 m distributed over 9 stations belonging to 3 tributaries (Aké, Biyeme, Ewoué) chosen on the basis of the effluents they receive. The R version 3.3.3 software was used to analyze the data. The Shannon diversity index, which ranges from 2.64 to 3.32, is indicative of a richness of floristic diversity in the Mfoundi lowlands. However, they are dominated by *Pennisetum purpureum* Schumach. (14.9%), *Commelina benghalensis* L. (9.3%) and *Echinochloa pyramidalis* (Lam.) Hitchc. & Chase (8.4 %). On each tributary studied, *Ipomoea aquatica* Forsk. abundantly appears at the intermediate level and disappears downstream. Other abundant species (*Hydrolea glabra* Schumach & Thonn, *Leersia hexandra* Sw. and *Pistia stratiotes* L.) have a fortuitous and evanescent appearance, approaching the above-mentioned specie but subservient only to the Aké tributary. These abundant species can give an indication of the quality of the water and guide the ecological restoration of degraded environments.

## Introduction

Human activities are one of the major causes of degradation of aquatic ecosystems (Vasquez and Favila, 1998, Priso et al., 2010, 2012 and 2014). In Cameroon, as in most developing countries, the streams in the urban areas are subjected to the discharge of untreated pollutants such as domestic sewage and industrial effluents, which degrade water quality while modifying the biodiversity of the ecosystems that constitute them (Winter and Duthie, 1998; Beyene et al., 2009). In the case of Yaounde city, industrial and agro-industrial activities develop on the Mfoundi watershed (Feudjeu, 2012). This leads to the accumulation of organic matter and increase in the basicity of water and suspended matter, which make Mfoundi a polluted watershed (Leutche et al., 2009). Despite all the work done on Mfoundi, it appears that the Mfoundi watershed is highly polluted (Ajeegah et al., 2006); this pollution of chemical and organic origin is the result of urban and industrial waste disposal in the city of Yaounde (Foto et al., 2011).

Previous studies on the quality of surface water in the city of Yaounde and more specifically the Mfoundi watershed have highlighted the presence of faecal contaminants (Foto, 1989; Kemka, 2000; Zébazé, 2000; Niyitegeka, 2001; Djuikom et al., 2009; Kuitcha et al., 2010; Nougang et al., 2011; Ajeegah et al., 2012; Anonyme, 2013). Based on physicochemical and bacteriological findings, Djuikom et al. (2009) concluded that the pollution of the Mfoundi watershed poses an increased risk of infection for the populations and that an urgent need to control the discharge of wastewater in this basin is required. Kuitcha et al. (2010) reported that the level of pollution in the Mfoundi watershed causes significant degradation of its waters and underlined the need to develop appropriate sanitation strategies. Ebang et al. (2012) have shown that epilithic diatoms are sensitive to variation in water quality of Mfoundi and can be used as bioindicators of the health status of Cameroon's urban water bodies. In this context, it is difficult with the current state of knowledge to define the specific characteristics of macrophytes in

the Mfoundi watershed. However, some plants such as *Echinochloa pyramidalis* have the capacity to purify wastewater discharged there (Fonkou et al., 2010; Djumyom et al., 2016). In addition, plants participate in the biotic and abiotic processes that occur in ecosystems and are therefore markers of environmental change (Ramade et al., 1984, Sauberer et al., 2003).

The urban environmental health can be determined by analysis of pollutants, by satellite methods or analysis of plant communities (Priso et al., 2000; Markert et al., 2003). The study of aquatic organisms provides an analysis that integrates the contamination of ecosystems because their presence or abundance reflects the successive environmental conditions that have occurred during their development (Jüttner et al., 1996; Soininen 2002; Beyene et al., 2009). The objective of this study is to show the influence of urban pollutant released from of various origins on the distribution of aquatic macrophytes and the pollution gradient of three Mfoundi tributaries.

## Materials and methods

### Study area

The study was carried out in Yaounde, the political capital of Cameroon, situated between 3° 30 and 3° 58 north latitude and 11° 20 and 11° 40 east longitude (Santoir and Bopda, 1995). This city is located on the southern plateau of Cameroon whose average altitude is 760 m (Abossolo et al., 2015). Yaounde is under the influence of a particular equatorial climate called "Yaounde climate" characterized by average rainfall of 1564.7 mm/year, an average temperature of 23.5° C and the annual thermal amplitude of 2.4 (Abossolo et al., 2015). Four seasons of unequal importance, varying in duration from one year to another, are observed, and are distributed as follows: a long dry season (mid-November to mid-March), a short rainy season (mid-March to mid-June), a short dry season (mid-June to mid-August) and a long rainy season (mid-August to mid-November). Only the Northwest and West

outskirts of Yaounde have few semi-deciduous forest relics with *Sterculiaceae* and *Ulmaceae* (Letouzey, 1985). In downtown and the pericentral area, rapid urbanization has decimated the existing forest, the greenery is visible swamp vegetation, fields and public gardens. Its base consists essentially of metamorphic and crystalline formations (Vicat and Bilong 1998 cit. Ebang et al., 2012) that give acidic soils with a pH below 6. The hydrographic network consists of Mfoundi and tributaries that irrigate almost the entire city. Mfoundi rises northwest of the city of Yaounde, mountain tops and Febe Mbankolo (1100 m), through the capital and join Mefou by the Borough of Afan-Oyoa. It drains 11 main tributaries: Tongolo, Ntem, Ebogo, Ewoue, Aké, Nkié, Odza (left bank) and Abiergué, Mingoa, Olézoa, Biyemé (right bank). Preliminary work has been done on the 11 tributaries of Mfoundi. The purpose is to list the types of waste and discards observed along the course of the tributary. At the end of this preliminary work, 3 tributaries of Mfoundi were selected (Fig. 1) according to the origins of the effluents they receive:

- The Aké tributary located on the left bank of the Mfoundi watershed where the industries are concentrated (the breweries of Cameroon, the Cameroonian union of breweries, the Coca Cola factory, the SODECAO (cocoa development company) and the SCDP (Cameroonian company of petroleum depots)). Its length is 5.3 km. This tributary receives chemical and organic pollutants of industrial origin;
- The Biyeme tributary located on the right bank of the Mfoundi watershed. Its length (11.6 km) makes it the most important tributary of the Mfoundi watershed (Foto et al., 2011). This tributary receives chemical and organic pollutants of domestic origin. However, its downstream part located in the Ahala district is considered here as the reference site given its position and its distance from all sources of pollution upstream of this tributary;
- Tributary Ewoué located on the left bank of the catchment basin of Mfoundi. Its length is 3.2 km. It is the only tributary of Mfoundi that is located near a market. Therefore, it receives a chemical and organic pollution of various origins.

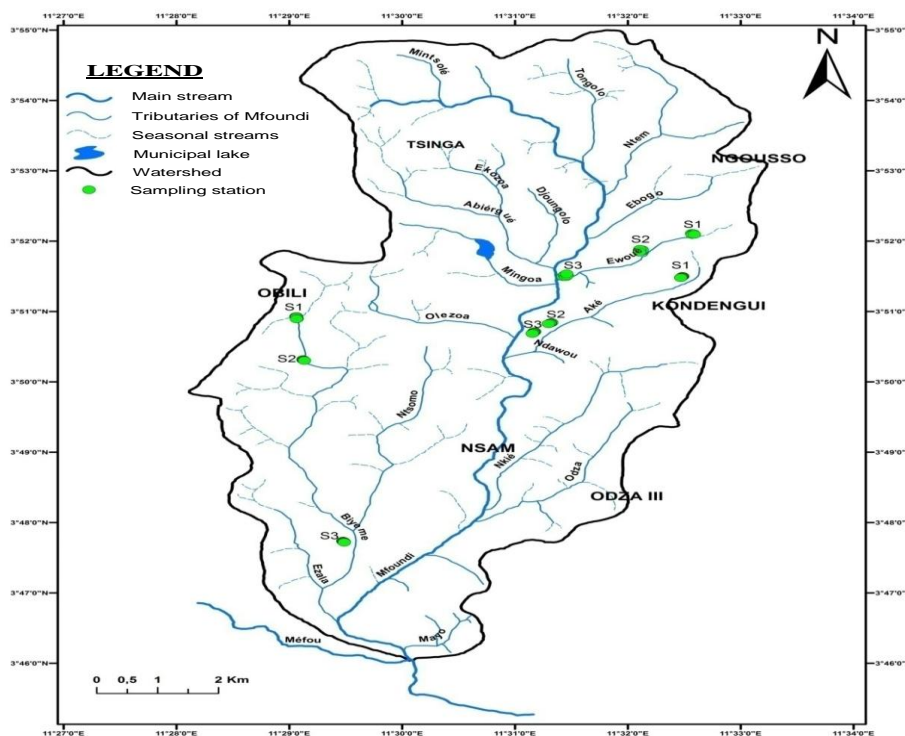


Fig. 1: Location of the 9 sampled stations on the 3 tributaries of the Mfoundi watershed.

## Data collection

Following the longitudinal gradient of each tributary, 10 plots of 5 m x 5 m distributed at regular intervals (5 m) were defined upstream, at an intermediate level and downstream of the tributary before its meeting with Mfoundi. These 3 points (upstream, intermediate and downstream) were considered as sampling stations or sites on each tributary made up a total of 90 plots, or 30 plots per tributary. The floristic survey was carried out during the long dry season and the big rainy season and was based on the identification of all macrophytes present in the sampled plots. Unidentified species in the field were harvested, labeled, pressed and dried; their identification was done by comparison of samples with the specimens of the National Herbarium of Cameroon. According to the average recovery rate of species, each of these species is affected by a Braun-Blanquet abundance-dominance coefficient (Masens, 1997). This coefficient, which varies from + to 5, is distributed as follows:

- + = simple presence, corresponding to an average recovery of 0.5;
- 1 = recovery of 1 to 5%, corresponding to an average recovery of 3;
- 2 = recovery of 5 to 25%, corresponding to an average coverage of 15;
- 3 = recovery of 25 to 50%, corresponding to an average recovery of 37.5;
- 4 = recovery of 50 to 75%, corresponding to an average recovery of 62.5;
- 5 = recovery greater than 75%, corresponding to an average recovery of 87.5.

## Data analysis

The floristic data were analyzed using the "Biodiversity R" package, software R version 3.3.3 (Kindt and Coe, 2005). Diversity indices such as Pielou and Shannon were calculated for the floristic characterization of the different stations. In addition

to the Sorensen similarity index, the floristic affinities between the different stations were assessed using a Principal Component Analysis.

The average recovery rate (RM) of a species in a given environment, which is the average percentage of occupancy of this specie in each station, was calculated using the dominance coefficient of abundance with the formula:  $RM_i = (R_i / \text{total number of records})$  where  $R_i$  is the total recovery of specie  $i$  in the medium.

The presence index ( $P_i$ ) corresponds to the average recovery of species  $i$  on the total recovery of individuals.  $P_i = RM_i / \sum RM$ .

The Shannon-weaver index ( $H'$ ) makes it possible to evaluate the floristic diversity. This index varies according to the average recovery of each species and the number of species present. It is high because a large number of species participate in land occupancy. It is expressed in bits per individual (Frontier et al., 2008; N'da et al., 2008). The formula used is as follows:  $H' = - \sum p_i \log_2 p_i$ ;  $P_i$  is the relative proportion of the average recovery of species  $i$  (values between 0 and 1).

The Pielou equilibrium ( $J$ ) makes it possible to measure the equidistribution of the species of the stand with respect to an equal theoretical distribution for all the species. It is obtained by the formula:  $J = H' / \log_2 S$ ;  $S$  is the number of species present. The  $J$  index tends to 0 when there is dominance of a single species and to 1 when a maximum of species participates in the recovery (Frontier et al., 2008; N'da et al., 2008).

The similarity index of Sorensen makes it possible to assess the floristic affinity between two ecological environments. It is greater than 50% when there is floristic affinity between two environments and less than 50 % otherwise. It is calculated by the following formula:  $CS = (2 c/a + b) \times 100$  with ( $a$  = number of species of medium A,  $b$  = number of species of medium B and  $c$  = number of species common to both ecological environments).



## Results

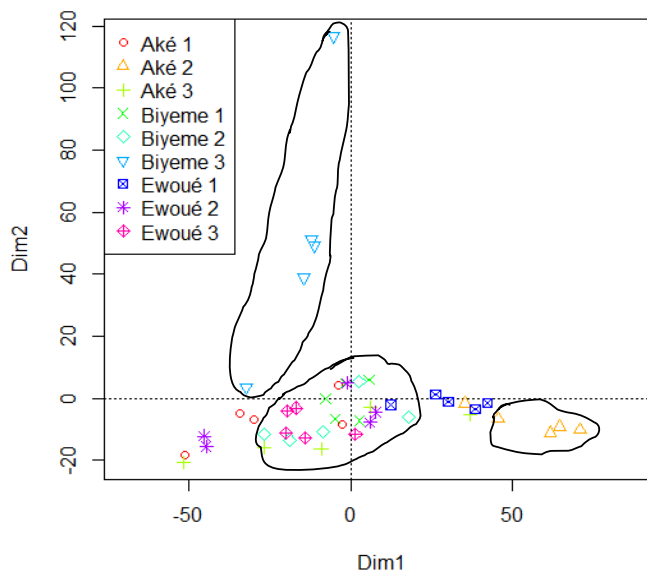
### Specific richness

A total of 135 species in 102 genera and 44 families were inventoried on the 3 tributaries. The most diverse families in terms of species richness are *Asteraceae* (16 species), *Poaceae* (12 species) and *Euphorbiaceae* (10 species). The most abundant *Asteraceae* are: *Tithonia diversifolia* (39.39%), *Struchium sparganophora* (21.81%) and *Acanthospermum hispidum* (14.80%). The most abundant *Poaceae* are: *Pennisetum purpureum* (43.56%), *Echinochloa pyramidalis* (24.56%) and *Panicum maximum* (12.33%). The most abundant *Euphorbiaceae* are: *Ricinus communis* (49.38%), *Phyllanthus amarus* (21.39 %) and *P. acidus* (8.23%).

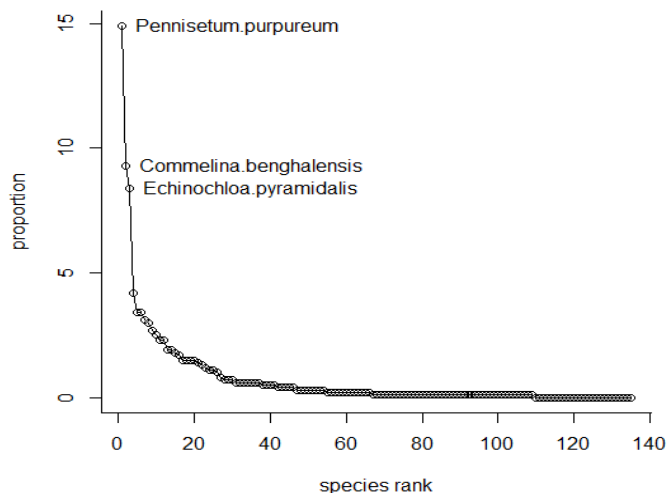
The Principal Component Analysis represented in Fig. 2 shows 3 plant associations according to the 3 groups observed:

- The first group, positively correlated along the ordinate axis consists solely of the site of Biyeme 3 which is the control site. It moves away completely from all the other stations. The abundant species present only in this site are: *Costus afer*, *Mitragyna stipulosa*, *Nymphaea lotus*, *Rhynchospora corymbosa* and *Setaria megaphylla*.
- The second group correlated positively along the abscissa axis consists only of the Aké 2 site. *Hydrolea glabra*, *Leersia hexandra* and *Pistia stratiotes* are 3 abundant and exclusive species at this station which is located at an intermediate level of the Aké tributary. *Ipomoea aquatica* is another species of this station that is also abundant only in stations that are intermediate to these 3 tributaries (Table 1). These abundant species, which appear abundantly only at the intermediate level of the tributaries, may have an indication of the quality of the water.
- The third group occupying the center of the axes consists of the rest of the other stations. The abundant species of this group are:

*Commelina benghalensis*, *Echinochloa pyramidalis*, and *Pennisetum purpureum*. Fig. 3 shows these species according to their abundance in the whole of the floristic inventory.



**Fig. 2:** Principal Component Analysis showing floristic affinities between sites.



**Fig. 3:** Curve showing 3 most abundant species in the inventory according to their proportion.

### Diversity index

The species richness decreases from upstream to downstream on the affluent Aké and Ewoué. The

opposite trend was observed on the Biyeme tributary, where it is stable from upstream to intermediate point and then increases downstream

(Table 2). The Aké 3 station has the smallest number of species (28 species) while the Biyeme 3 station has the highest species richness (52 species).

**Table 1.** Percentage of abundant species in the 3 tributaries.

| Espèces                        | Sites | Aké  |      |      | Biyeme |      |      | Ewoué |      |      |
|--------------------------------|-------|------|------|------|--------|------|------|-------|------|------|
|                                |       | 1    | 2    | 3    | 1      | 2    | 3    | 1     | 2    | 3    |
| <i>Acroceras zyzanioides</i>   |       | 0.06 |      |      |        | 1.62 | 13.0 |       | 1.53 |      |
| <i>Alternanthera ficoidea</i>  |       | 1.73 | 0.38 | 7.08 | 5.12   | 4.42 | 0.50 | 6.16  | 4.85 | 1.69 |
| <i>Alternanthera sessilis</i>  |       | 1.73 | 3.88 | 1.77 | 3.56   | 3.98 | 1.17 | 3.56  | 6.94 | 1.64 |
| <i>Amaranthus esculentus</i>   |       | 1.40 | 0.77 | 0.04 | 3.12   | 4.16 |      | 0.33  | 0.05 | 1.09 |
| <i>Commelina benghalensis</i>  |       | 8.75 | 7.12 | 5.09 | 12.2   | 8.76 | 7.34 | 14.9  | 12.2 | 9.69 |
| <i>Costus afer</i>             |       |      |      |      |        |      | 4.17 |       |      |      |
| <i>Echinochloa pyramidalis</i> |       | 2.81 | 16.7 | 11.5 | 4.05   | 5.70 | 0.33 | 23.5  | 4.85 | 8.59 |
| <i>Hydrolea glabra</i>         |       |      | 17.5 |      |        |      |      |       |      |      |
| <i>Ipomoea alba</i>            |       | 0.66 | 0.38 | 3.10 | 3.11   |      | 0.33 |       |      | 6.01 |
| <i>Ipomoea aquatica</i>        |       |      | 6.35 |      |        | 11.2 |      | 1.47  | 3.93 |      |
| <i>Ipomoea batatas</i>         |       | 4.87 | 0.04 |      | 4.67   | 4.47 | 5.34 | 7.07  | 1.27 | 4.17 |
| <i>Justicia secunda</i>        |       | 4.21 |      |      | 8.46   | 0.57 |      | 1.41  |      |      |
| <i>Leersia hexandra</i>        |       |      | 5.19 |      |        |      |      |       |      |      |
| <i>Ludwigia abyssinica</i>     |       |      | 4.62 | 0.66 | 0.04   | 1.14 | 1.37 |       |      |      |
| <i>Luffa aegyptiaca</i>        |       | 3.74 | 0.96 | 2.21 | 1.33   | 5.87 |      |       |      | 3.57 |
| <i>Mitragyna stipulosa</i>     |       |      |      |      |        |      | 4.00 |       |      |      |
| <i>Nymphaea lotus</i>          |       |      |      |      |        |      | 4.67 |       |      |      |
| <i>Panicum maximum</i>         |       | 2.07 | 6.15 | 8.42 | 4.00   | 2.63 | 3.50 | 3.68  | 1.84 | 4.57 |
| <i>Pennisetum purpureum</i>    |       | 37.8 | 3.08 | 18.4 | 6.23   | 15.5 | 9.18 | 3.45  | 29.8 | 22.3 |
| <i>Pistia stratiotes</i>       |       |      | 3.46 |      |        |      |      |       |      |      |
| <i>Polygonum lanigerum</i>     |       |      | 3.85 | 1.33 |        |      |      |       | 6.89 | 1.49 |
| <i>Polygonum salicifolium</i>  |       |      | 3.19 | 2.66 |        |      | 5.00 |       |      |      |
| <i>Ricinus communis</i>        |       |      |      |      | 1.78   |      |      |       |      | 3.98 |
| <i>Rhynchospora corymbosa</i>  |       |      |      |      |        |      | 7.17 |       |      |      |
| <i>Setaria barbata</i>         |       | 1.80 | 0.96 |      | 4.50   | 2.06 |      | 0.84  | 1.78 | 0.74 |
| <i>Setaria megaphylla</i>      |       |      |      |      |        |      | 4.17 |       |      |      |
| <i>Struchium sparganophora</i> |       |      | 1.15 | 2.66 |        |      | 8.01 |       |      |      |
| <i>Tithonia diversifolia</i>   |       |      |      | 9.74 | 4.45   |      | 0.16 |       | 2.96 | 10.5 |
| <i>Xanthosoma mafaffa</i>      |       | 2.14 | 0.11 | 2.66 | 4.67   | 0.78 |      | 3.34  | 2.96 | 0.49 |
| <i>Zehneria scabra</i>         |       | 3.94 |      | 8.86 | 2.23   | 1.09 |      | 1.98  | 3.16 | 3.23 |

1 = upstream; 2 = intermediate; 3 = downstream.

**Table 2.** Diversity index in the different study stations.

| Tributaries | Stations | Specific richness | Shannon index | Pielou equitability |
|-------------|----------|-------------------|---------------|---------------------|
| Aké         | 1        | 50                | 2.73          | 0.698               |
|             | 2        | 45                | 2.92          | 0.766               |
|             | 3        | 28                | 2.77          | 0.833               |
| Biyeme      | 1        | 46                | 3.32          | 0.866               |
|             | 2        | 46                | 3.15          | 0.824               |
|             | 3        | 52                | 3.19          | 0.808               |
| Ewoué       | 1        | 50                | 2.92          | 0.745               |
|             | 2        | 43                | 2.64          | 0.701               |
|             | 3        | 46                | 2.87          | 0.751               |

The Shannon diversity index increases from upstream to downstream on the Aké tributary and decreases from upstream to downstream on the Biyeme and Ewoué tributary. The highest Shannon index (3.32) is obtained at Biyeme 1 (Etoug-ebe) and the lowest (2.64) at Ewoué 2 (Corneillet). Table 2 shows that Pielou

equitability varies from 0.698 (Aké 1) to 0.866 (Biyeme 1). The similarity coefficients of Sorensen between the control station or Biyeme 3 and the other stations vary from 21.35 % to 39.17 %, so they are all less than 50% (Table 3). These are the lowest similarity indices observed in Table 3.

**Table 3.** Coefficient of similarity of Sorensen between the different stations.

|                 | Aké 1 | Aké 2 | Aké 3 | Biyeme 1 | Biyeme 2 | Biyeme 3 | Ewoué 1 | Ewoué 2 | Ewoué 3 |
|-----------------|-------|-------|-------|----------|----------|----------|---------|---------|---------|
| <b>Aké 1</b>    | 100   |       |       |          |          |          |         |         |         |
| <b>Aké 2</b>    | 42.10 | 100   |       |          |          |          |         |         |         |
| <b>Aké 3</b>    | 46.15 | 49.31 | 100   |          |          |          |         |         |         |
| <b>Biyeme 1</b> | 62.50 | 46.15 | 59.45 | 100      |          |          |         |         |         |
| <b>Biyeme 2</b> | 60.41 | 50.54 | 51.35 | 65.21    | 100      |          |         |         |         |
| <b>Biyeme 3</b> | 29.41 | 39.17 | 37.5  | 28.57    | 32.65    | 100      |         |         |         |
| <b>Ewoué 1</b>  | 55.44 | 45.83 | 40.50 | 59.79    | 61.85    | 21.35    | 100     |         |         |
| <b>Ewoué 2</b>  | 63.82 | 49.43 | 50    | 60       | 64.44    | 27.08    | 61.05   | 100     |         |
| <b>Ewoué 3</b>  | 63.91 | 52.74 | 50.66 | 60.21    | 55.91    | 32.32    | 59.18   | 68.13   | 100     |

## Discussion

In general, species such as *Commelina benghalensis*, *Echinochloa pyramidalis* and *Pennisetum purpureum* are the most abundant on the entire floristic inventory. *Commelina benghalensis* was particularly abundant in all sites. This goes in line with the fact that, *Commelinaceae* have already been recognised in Cameroon as an indicator of pollution of aquatic environments (Priso et al., 2000).

Many studies have also shown that *Poaceae* are among the most polluting plants (Anoliefo et al., 2008). The different sites studied may be polluted to different types or degrees of pollution since they do not all have the same floristic composition though they all belong to the Mfoundi watershed.

This difference between the sites is observed on the Principal Component Analysis which divides them into 3 groups. The first group formed solely by the control site (Biyeme 3) revealed the species that characterize it. Indeed, the similarity coefficient of Sorensen shows that the control site (Biyeme 3) does not show any marked floristic similarity with all the other stations. The dissimilarity of this flora is characterized by a Sorensen index of less than

50%. The smaller the Sorensen similarity index, the less similar the sites are (Ngueguim et al., 2010). This can be explained by the fact that the control station located on the Biyeme tributary downstream of the Mfoundi watershed is not close to the houses, though individuals often come to bathe while all the other stations are upstream of this watershed. These stations located upstream of the Mfoundi watershed receive the majority of discharges of urban origin. The second group is formed only by the Aké 2 station which is located near the industries. This site receives industrial and domestic wastewater; effluents from laundries, car garages and wastewater from the cleaning of plastic bottles. *Hydrolea glabra*, *Leersia hexandra* and *Pistia stratiotes* are the abundant and characteristic species of this station. *Ipomoea aquatica* is an abundant species of this station that also appears only at the intermediate level of the other two tributaries.

Ogbo et al. (2009) report that the high abundance of a species in an altered site may give an indication of the resistance of the plant to this polluting cocktail and provide information on its phyto-remediation potential. Thus, *Pistia stratiotes* is among the most appropriate plants for phyto-remediation in the tropics (Fonkou et al., 2002) and *Ipomoea aquatica*

is often used in combination with other plants (*Enydra fluctuans* and *Commelina nudiflora*) in the treatment of wastewater (Nya et al., 2002). Fonkou et al. (2005) identified the presence of heavy metals (cadmium, copper, zinc, lead) in all the compartments of two fish ponds in Yaounde: water, sediments, but also plants (*Cyperus papyrus*, *Enydra fluctuans*, *Ipomoea aquatica* and *Echinochloa pyramidalis*). These plants have been tested in artificial swamps for sewage treatment. The third group consists of all the other stations. These sites receive domestic wastewater from small businesses and other various human activities including latrines-cannons that contaminate and degrade the quality of water. *Commelina benghalensis*, *Echinochloa pyramidalis* and *Pennisetum purpureum* are the species that thrive in these stations. Moreover, Diop (2010) has shown that interspecific competition can significantly influence the distribution of vegetation because if one species settles first, its growth and proliferation may inhibit the growth of another secondary arrival.

Barendregt and Bio (2003) believe that water, light, temperature and nitrates are the factors that influence macrophytic growth in small streams. The species richness decreases from upstream to downstream on the Aké and Ewoué tributaries. According to Ajeagah et al. (2006), there is a large impact of human activities and intensification of pollution from upstream to downstream of the Mfoundi watershed. All the waters upstream of the Mfoundi watershed cross a swampy area with its phyto-purifying character before arriving at the Biyeme 3 control station. The evolution of the species richness on the Biyeme tributary which is the reverse of that of Aké and Ewoué tributaries corroborate that of Priso et al. (2012) on the Kondi River in Douala as well as that of Dibong and Ndjouondo (2014) on the Kambo River in Douala. In all the stations studied, the Shannon diversity indices are between 2.64 and 3.32; equitability of Piéou is between 0.698 and 0.866. This means that all the stations are diversified and that a maximum of species participates in the recovery of the surface.

## Conclusion

This study has highlighted pollution indicative plants in a few tributaries of the Mfoundi watershed in the city of Yaounde. *Commelina benghalensis*, *Echinochloa pyramidalis* and *Pennisetum purpureum* are the most abundant species in this polluted watershed. In addition to these species, *Hydrolea glabra*, *Ipomoea aquatica*, *Leersia hexandra* and *Pistia stratiotes* have characterized the sites receiving industrial pollution (mainly the Aké 2 site on the Aké tributary). On the other hand, the reference site furthest from this urban pollution is characterized by: *Costus afer*, *Mitragyna stipulosa*, *Nymphaea lotus*, *Rhynchospora corymbosa* and *Setaria megaphylla*. Abundant species in polluted sites can be tested in artificial swamps for sewage treatment.

## Conflict of interest statement

Authors declare that they have no conflict of interest.

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