International Journal of Environmental Engineering Science and Technology Research
Vol. 5, No. 1, July 2017, pp. 1-12, ISSN: 2326 - 3113 (Online)
Available online at http://ijestr.org/

Research article

Purifying role of *Eichhorniacrassipes*in Nokoue lake, Republic of Benin

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Abstract:

The impact of pollution of Lake Nokoue and its adverse consequences on water and fishery resources has been studied. The characterization of this pollution was to measure conventional parameters of surface water quality, nutrient salts (nitrite, nitrate, ammonium, and phosphate) and lead residues in sediments and aquatic plants, also to determine the values of these monitoring indicators for a better purification yield in a natural environment. The immediate consequence of eutrophication on Nokoue Lake is the proliferation of water hyacinth (*Eichhorniacrassipes)* when salinity conditions are favorable (salinity <7 ‰). The low levels of dissolved oxygen would be due not only to the effects of *Eichhorniacrassipes*(*EC*) during low-water periods, but also to the organic loads of the lake discharge. Bio Concentration Factor (BCF) is 1/3 for living hyacinth, sedimentary lead is then three (3) times higher than that accumulated in water hyacinth. The threat posed by the proliferation of *Eichhorniacrassipes* could be transformed into an opportunity to de-pollute the environment during the periodof high salinity and high level water.

Keywords: *Eichhorniacrassipes*, purification, lead, nutrient salts, Lake Nokoué

1- Introduction

The qualities of plant scrubbers with respect to toxic metals and nutrients salts have been the subject of various studies (Hansen et al., 1971; Gel et al., 1990; Lahlali et al., 2007) and the choice of lead among toxic metals is justified in this study mainly by its ubiquity. The main source of lead in the environment is atmospheric deposition from industrial processes and transport. Different studies have reported very close links between nutrient salts and the proliferation of algae and other aquatic plants [Hancock, et al., 1991; USEPA., 1988]. Methods for removing the pollutant load from wastewater have been developed by different researchers for the preservation and regeneration of the quality of the receptor media either by macrophytes or by microorganisms [USEPA, 1988, Dagnoet al., 2007, Fox et al., 2008]. Plants uses a certain amount of nitrogen and phosphorus during growth, and can in some cases consume and concentrate some heavy metals in their stems and leaves [Gajalakshmi et al., 2002, McVea, et al., 1975]. On the other hand, removing phosphorus from water using floating plants such as lenticules (*Lemna sp.*) [Hancock, 1991] and water hyacinths (*Eichhorniacrassipes*) has been proved effective in several cases [USEPA, 1988]. The case of metals is generally studied in controlled environments whose composition is controlled. However, the literature reports very close links between salinity, organic material and the accumulation of metals by aquatic species [Sankaret al., 2005, Center, 2002, Gopal,1987.,Jijakli, 2007]. In general, when metals are complexed with dissolved organic material and / or inorganic ions, lower degrees of bioavailability and toxicity of aquatic environments are observed [Sankar et al., 2005, Center et al. 2002]. This real-world study aims to establish these links and propose in situ approaches for improving the quality of aquatic environments and water resources exploited for human consumption.

2 Materials and methods

2.1 Presentation of the Nokoue Lake

Nokoue Lake is located on three departments: Littoral, Atlantique and Oueme. Covering approximatively160 km², it is located in the lower valley of River Oueme and river Sô. It is limited by the communes of Cotonou, Abomey-Calavi and Sô-Ava in the department of the Atlantic - Littoral and Porto-Novo in the department of Oueme - Plateau, communes from which it receives the main contributions of pollutants. Among them, the commune of Sô-Ava has the peculiarity of being made up of lacustrine villages which discharge their waste into this watercourse. The climate of southern Benin is of the subequatorial type characterized by two alternating rainy seasons with two dry seasons.

The hydrographic regime shows streams coming from the northern part of the country such as the most important river Oueme of the tributaries of NokoueLake.

2.2Materials and water quality monitoring indicators

2.2.1 Vegetable material: *Eichhorniacrassipes*

*Eichhorniacrassipes*is a plant described by several authors as being the worst of all aquatic plants. It develops abundantly in different areas of NokoueLake. The systematics of water hyacinth can thus be presented as: Kingdom: Plantae; Sub-kingdom: Tracheobinota; Upper division: Magnoliophyta; Class: Liliopsida; Subclass: Lines and Genus: *Eichhornia*

2.2.2 In situ Sampling campaigns and parameter measurement

Six sampling campaigns were carried out on three stations with two stations in the lake villages (Ganvie and Sô Ava) colonized by *Eichhorniacrassipes* and a station located in the middle of the lake as a control site.

2.3 Analytical methods

Organic carbon levels of sediment and plant material were determined using the WALKEY and BLACK method. This method consists of oxidizing the organic carbon with potassium dichromate ($K_2Cr_2O_7$) in the presence of sulfuric acid (H_2SO_4).

The Zambelli reagent method was used for the determination of nitrites, ammonium molybdate and Nessler reagent (alkaline potassium iodomercurate), respectively, for orthophosphate and ammonium ions. As for nitrate ions, they were determined by the sodium salicylate method. Total kjeldahl nitrogen was determined after mineralization of the organic ammonium nitrogen displaced by a sodium hydroxide solution which is then entrained by a steam and then assayed colorimetric method based on Nessler method [Rodier,1984].

Samples of sediment and *Eichhorniacrassipes*have been treated according to H / NF EN ISO 15587-1 and 2 for the mineralization of lead (Pb) in aqua regia (HCl + HNO_3: 3/1). The mineralized product obtained was filtered and up to 50 ml was taken with distilled water and then analyzed with the atomic absorption spectrometer AAS-flame type GTA 110 VARIAN at the wavelength λ = 217 nm

3 Results and discussion

3.1 Physico-chemical characterization of the environment

Physical parameters as temperature, pH, salinity, dissolved oxygen and conductivity of water were presented in table 1. This table shows that the conductivity is low in Sô-Ava and Ganvie stations. The relatively high values of conductivity are not only explained by a salt intrusion from Atlantic Ocean into the lake because even at zero salinity, these values are at the same order of magnitude in the same period (Table 1). On the other hand, high salinity levels in March and May shows saline intrusion, as those values are very close to marine salinity. The mean of the salinity, which is 9.61 ‰ obtained overall, is not representative of the study environment over the period of study because the standard deviation is 11.65 which is quite high. This is due to the large variations observed during the seasons, i.e. high values during the low-water period and very low values close to 0 during the flood periods. This would explain the appearance of water hyacinth on these sites during this period (September and October) because *Echhorniacrassipes*is controlled not only by salinity but also by the limiting factor like phosphorus. The salinity levels from which this phenomenon is observed as well as the periods of disappearance will be highlighted in the rest of this study. On the other hand, this result would explain the low levels of dissolved oxygen levels, characteristic of a medium prone to an oxygen deficit that could compromise aquatic life, especially since organic matter is available because it is mineralized. This can be caused by the high organic loads due to discharges and detritus from the bottom of the lake as shown by the exchanges of total organic matter and organic carbon (Corg) on the graphs of material exchange.

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Table 1: Physical parameters of water in the Nokoue Lake

Months	*Locality*	*Temp (°C)*	*pH*	*Salinity (mg/L)*	*Oxy_dis (mgO_2/L)*	*Conductivity (µS/Cm)*
	Ganvie	28	6,79	0	2,8	1394
	Soava	28.1	6.72	0	2.3	1360
Sept	Mlak	28.4	7.26	0	3.6	1360
	Ganvie	30.1	6.5	0	3.7	1341
	Soava	30.5	6.3	1	2.6	1341
Oct	Mlak	30.8	7.6	0	3.8	1351
	Ganvie	29.0	7.15	0	3.1	1232
	Soava	28.5	7.2	2	3.0	1589
Dec	Mlak	28.5	7.8	5	4.2	1589
	Ganvie	29.0	6.63	15	3.5	1270
	Soava	29.0	8.32	15	3.2	1380
Mar	Mlak	29.7	7.72	33	3.3	1275
	Ganvie	30.0	7.32	25	3.6	1590
	Soava	29.5	7.18	19	3.2	1329
May	Mlak	29.0	5.72	32	4.3	1846
	Ganvi	30.0	7.78	5	3.6	1270
	Soava	29.0	6.87	2	2.8	1380
Jun	Mlak	29.0	7.72	19	4.3	1275

Legend:	***Symbol***	**Temp:**	**Oxy_dis:**	**Mlak:**
	Signification	Temperature	dissolved oxygen	Middle of the lac

3.2 Nutrient elements

3.2.1 Nutrient salts dissolved in water

The nutrient salts parameters that were measured are nitrite, nitrate, ammonium and phosphate ions in water samples and in the aquatic plant samples (*Echhorniacrassipes*). Results in tables 2 and 3 show that nutrient salts are lowers in water and water hyacinth. There was no presence of water hyacinth in March and May.

Table 2: Concentrations of nutrient salts in water in Nokoue Lake

Parameters /Station	*Period (months)*						*Mean*	*ET*
	Sept	*Oct*	*Dec*	*March*	*May*	*June*		
Nitrite (mg/l)								
Ganvié	0.31	0.42	0.21	0.01	0.02	0.48	0.24	0.20
Sô Ava	0.46	0.32	0.17	0.01	0.01	0.56	0.26	0.23
Mlak	0.04	0.37	0.16	0.01	0.01	0.10	0.12	0.14
Nitrate (mg/l)								
Ganvie	14.44	2.35	9.00	1.40	3.20	traces	6.08	5.53
Sô Ava	14.08	3.19	8.00	1.10	1.80	traces	5.63	5.44
Mlak	12.14	1.42	2.10	0.90	1.70	traces	3.65	4.76
Ammonium (mg/l)								
Ganvie	0.13	0.03	traces	1.75	4.60	0.20	1.12	1.53
Sô Ava	1.28	0.03	traces	1.25	3.80	0.40	1.13	1.44
Mlak	0.38	0.03	traces	2.00	4.00	0.40	1.13	1.68
Phosphates (mg/l)								
Ganvie	1.27	0.04	1.72	1.40	1.07	0.19	0.95	0.68
Sô-Ava	1.27	0.04	traces	1.45	0.47	0.15	0.68	0.65
Mlak	0.38	0.80	traces	7.65	0.41	0.17	1.88	3.23

Nutrient salts are determining factor in the proliferation of aquatic plants while phosphate salts are limiting factors in a natural aquatic environment. It is then necessary to establish the favorable conditions from the point of view of the nutrients in order to define the ranges of concentrations favorable to a normal development of the plant. From the analysis of results in Table 2, we can say that the lowest values of nitrite and nitrate ions are obtained in March and May, a period of high salinity, unlike the ammonium ions which show maximum values in March.

The periods of March and May are marked by a total absence of water hyacinth at the various sites. On the other hand, the highest value of the salts of nitrite and nitrate is recorded in June at Sô Ava. It is worth to note that the month with the lowest nutrients is December. In general, the values of nitrite in September, October and June are above average. The same trends can be observed for nitrate ions except that in June, there is almost no nitrate, more preferably nitrogen is essentially in reduced form Nitrite or organic nitrogen with relatively high values of nitrite in this month at the colonization sites of water hyacinth. The high levels of nitrate obtained in September, coinciding with the end of the rainy season in the northern part, can be explained by the high intakes of this salt by the waters of the north, which have had to leach agricultural areas on their journey.

Phosphate peaks were also observed in March at sites colonized by water hyacinth with a maximum value of 7.65 mg / L in the middle of the lake. The minimum value is 0.04 mg / L for an average of 1.16 mg / L. These relatively high concentrations of phosphorus in the lake environment could be attributed to several sources, the most important being northern waters likely to be loaded with phosphorous nutrients of agricultural origin; Other sources may be domestic releases of sludge and detergents and runoff from riparian communities.

As for ammonium ions, they find their peak concentration in the month of May in the absence of living water hyacinth and the minimum for these ions is obtained in October with an average of 1.6 mg / L and a coefficient of variation of 1.82 showing a strong dispersion of values. These high values of the ammonium ion in May could result from the reduction of nitrates due to the anaerobic conditions in the lake due to fall in the number of the water hyacinths and their mineralization.

In sum, phosphorus did not act as a limiting factor for the development of aquatic plants, contrary to the literature because the water hyacinth population is either non-existent or low density where it exists, as soon as the conditions of salinity (high salinity) are not conducive to their development.

In addition, the disappearance of the *Eichhorniacrassipes* vegetation cover seems to coincide with the disappearance of oxidized forms of nitrogen in favor of reduced forms, from December to May for nitrite ions and June for nitrate ions. The opposite phenomenon occurs with the ammonium and phosphate ions disappearing with the vegetation cover more noticeably in October and June.

3.2.2 Nutrient salts in the *Eichhorniacrassipes* plant

The remark that emerges from Table 3 is that the water hyacinth disappeared from the lake in March and May at the two sites chosen on the lake.

Table 3: Concentrations of *EC* nutrient salts in Nokoue Lake

Parameters /Station	***Period (months)***						***Mean***	***ET***
	Sept	Oct	Dec	Mar	May	Jun		
Nitrite in water Hyacinth (mg/kg)								
Ganvié	0.02	9.50	No	No	No	0.20	2.44	4.70
Sô-Ava	0.02	1.30	No	No	No	1.30	0.65	0.70
Nitrate in waterHyacinth (mg/kg)								
Ganvié	0.10	8.00	0.3	No	No	No	2.80	4.50
Sô-Ava	0.20	20	0.2	No	No	No	6.80	11.00
Phosphate in waterHyacinth (mg/kg)								
Ganvié	0.02	1.30	2.60	No	No	15.00	4.61	5.80
Sô-Ava	0.01	1.30	2.80	No	No	26.00	7.59	11.00

Note: Where there is no value, there was no water hyacinth

The recovery of the plants will have the impact of depollution because the nutrient salts are recovered in a notable way but a law of variation or a correlation could not be established with the concentrations in the water. However, the literature reports a recovery of 80% compared to the values of nutrient salts in the environment. An assessment in a natural environment would not represent the actual situation because the inputs are not controlled. Nevertheless, the phosphate salts seem to be more accumulated in June to the point of strongly influencing the overall average according to the results obtained by Hillet al. (1997) and USEPA, (1988).

3.3 Relationship between sedimentary lead and lead in *EC*

Water hyacinth, a floating plant characterized by high invasive power and a growth rate of about 200 tons per hectare per year, makes this plant one of the apparent signs of eutrophication of water. This plant is known to grow in the presence of nutrients such as nitrates, nitrites, phosphates and heavy metals such as lead, cadmium, chromium etc. It is used for the purification of waste water by the lake. Different studies show that *Eichhorniacrassipes* found in these natural systems is used in diverse fields as feed livestock breeding, fishfarming, agriculture or renewable energies (USEPA, 1988;Dagnoet al., 2007; Fox et al., 2008) at Ganvie and at Sô-Ava. This would reflect a balance of exchange between the two components of the aquatic ecosystem. However, because the transfer factor (BCF) is 1/3 for living hyacinth, sedimentary lead is three (3) times higher than that accumulated in water hyacinth (Figure1). This relationship is much more verified at zero salinity in September and October (Figure2a and b).

As salinity increases, lead is increasingly concentrated in water hyacinth in comparison to thatthe sediment and water. Paradoxically, the dead hyacinth contained a little more lead than the living one (BCF> 1/3) according to

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Available online at http://ijestr.org/

the graphs in Figure2. However, the dry matter yields in *Eichhorniacrassipes* are lower than those in sediments while the organic carbon of water hyacinth is 5 to 6 times higher than that in sediments.

The lead content in the So-Ava water hyacinth remains almost constant and below the overall average throughout the sampling period, except at the beginning of September when we obtained 140 mg / kg. This result is in line with the previous one, which indicates a higher concentration in the dead water hyacinths compared to living ones. The simultaneous decrease in lead content in water hyacinth and sediments under the influence of salinity indicates that the transfer of lead is favored towards the water column. Thus, salinity increases the mobility of lead and especially its passage in the water column by the release of molecules of water by the water hyacinth. On the whole the curve reflects relatively high concentrations in the two compartments studied namely, sediments and *Eichhorniacrassipes*

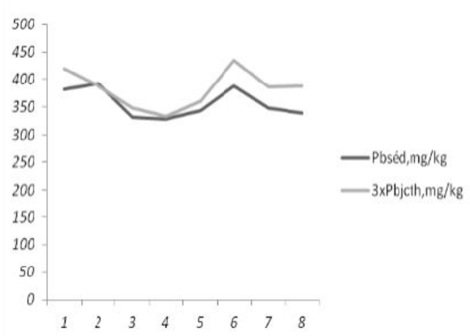

Figure 1: Evolution of lead in sediment and water hyacinth

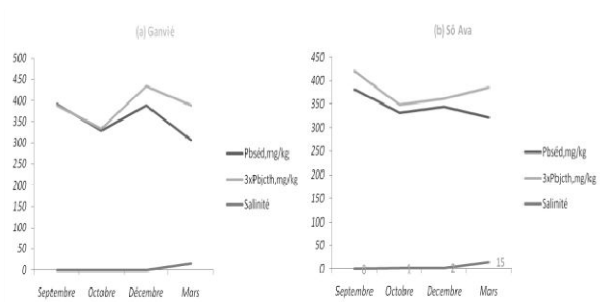

Figure 2: Evolution of lead levels in sediments and water hyacinth as a function of salinity
a) *inGanviéb) in So Ava*

Appeared that, salinity is the main factor controlling the proliferation of water hyacinth. The critical value of the salinity from which plant development stops is 7 mg / L according to the work of Lalèyé (1995) and the recommended period for the recovery of dead water hyacinths before releasing their contents, in particular lead and accumulated nutrient salts is located towards the end of February. For the lead, the recovery rate is about 33%, for the nutritional salts mores studies will have to be carried out.

3.4 Carbon exchange mechanism between sediments and *EC*

Salinity is the main factor that determines the presence of water hyacinth in the lake. At high salinity, in the months of March and May in particular, the hyacinth precipitates at the bottom of the water and covers the sediments (figure3) during which it increases the organic carbon content. During flooding, they proliferate but disappear as soon as the salinity exceeds 7 g / L (Lalèyè, 1995).

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The samples of water hyacinth analyzed have organic matter levels between 303 and 871 mg / g with an average of 569 mg / g of dry matter. The time variations are the same for the Ganvie and So-Ava samples. The high organic matter values are observed during the period from the end of October to the end of December for the water hyacinths of the two stations. A recovery of water hyacinth in June reduced organic carbon values to their levels from October to December.

Falling rates followed in March, with organic matter levels of 636 mg/g in Ganvié and 303 in So-Ava. These low rates observed in March were probably due to the decomposition and putrefaction state in which these plants were found during the sampling season. In March, the release of materials into the water column and sediments which exhibit variations in the opposite direction to plants was noted.

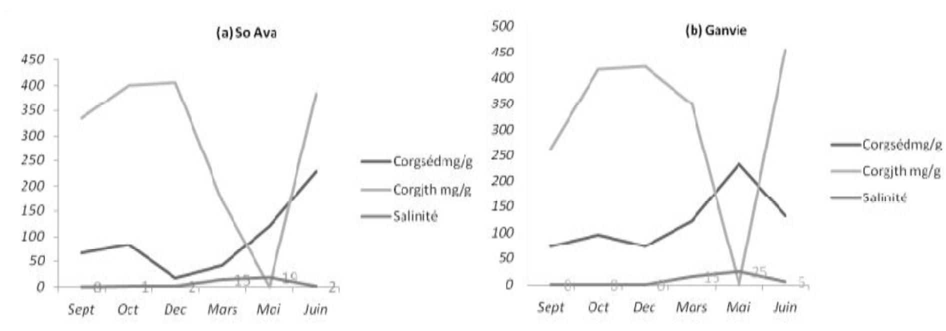

Figure 3: *Sedimentary organic carbon relationship - water hyacinth as a function of salinity*
a) In So Ava   b) inGanvié

The relative high value of the water salinity at these stations at this date (> 10 ‰) would explain these declines recorded in the plants and the increase in the sediments. It should be noted that, in March and May, no water hyacinth harvest could be carried out because the plant was totally absent from the water body at these sites. The critical period for the release of organic matter into sediments is between November and early March and the mineralization that accompanies these processes could increase the bioavailability of metals and thus the toxicity of the aquatic environment.

4 Conclusion and perspectives

The purifying properties of this plant in real environment are highlighted. The critical periods of collection of plant material were especially for the accumulated metals (lead), nutrient salts and organic matter is located between Novembers to early March. In water bodies, the main natural control factor for floating aquatic vegetation is salinity (<7mg / L). Conversely, the presence of aquatic plants influences the processes of transformation of nutrient salts with a significant effect on aquatic life.

Acknowledgments:

FinancialsupportwasprovidedbyTheInternational FoundationforScience(IFS).

REFERENCES

Center T.D., Hill M.P., Cordo H., Julien M.H. , 2002, Waterhyacinth, 4164p.

Dagno K., Lahlali R., Friel D., Bajji M., Jijakli M.H. 2007. «Synthèse bibliographique : problématique de la jacinthe d'eau, Eichhorniacrassipes, dans les régions tropicales et subtropicales du monde, notamment son éradication par la lutte biologique au moyen des phytopathogènes». Biotechnology, Agronomy, Society and Environment , 11 : 299311.

Fox L.J., Struik P.C., Appleton B.L., Rule J.H. , 2008, «Nitrogen Phytoremediation by Water Hyacinth (Eichhorniacrassipes (Mart.) Solms) ». Water Air and Soil Pollution, 194: 199207.

Gajalakshmi S., Ramasamy E.V., Abbasi S.A., 2002, «Highrate Composting vermicomposting of water hyacinth (Eichhorniacrassipes, Mart. Solms)». Bioresource Technology 83: 235239.

Geller, Gunther, Kleyn K., et LENZ A., 1990 1 «Planted soi1 filters" forwastewater treatment: the complex system planted soi1 filter, its components and their development » Pergamon Press, IAWPRC Conférence "Advances in water Pollution Control", pp. 161 -1 70 pages

Gopal B. 1987. «Water hyacinth »,. Elsevier, Amsterdam, The Netherlands. 471p. In :Dagno K., Lahlali R., Friel D., Bajji

Hancock, Jean. J. Etlakshime r, Budd havarapu, 1991, « Advanced wastewater treatment with lemna Technology Lernna Corporation », Compterendu de conference "Constructed wetlands for water quaiiiirnprovement- An international symposium-PensaslaFloride.", 21 pages.

Hansen K.L., Ruby E.G., Thompson R.L. 1971, «Trophic relationships in the water hyacinth community». Quarterly Journal of the Florida Academy of Science 34: 107113. In: EPPO European and mediterranean Plant Protection Organization 2008. , Data sheets on quarantine pests. Fiches informatives sur les organismes de quarantaine Eichhorniacrassipes. Bulletin OEPP/EPPO 38: 441449.

Hill G., Waage J., Phiri G. 1997, « The water hyacinth problem in tropical Africa», Proceedings of the first meeting of the International Water Hyacinth Consortium, 1819 March. Washington: World Bank. In: Dagno K.,

Lahlali R., Friel D., Bajji M., Jijakli M.H. 2007.« Synthèse bibliographique : problématique de la jacinthe d'eau, Eichhorniacrassipes, dans les régions tropicales et subtropicales du monde, notamment son éradication par la lutte biologique au moyen des phytopathogènes», Biotechnology, Agronomy, Society and Environment 11 : 299311

Lalèyè P., 1995. Ecologie comparée de deux espèces du Chrysichthys, poissons siluriformes (Clarotéidae) du Complexe lagunaire lac Nokoué-lagune de Porto-Novo au Bénin. Thèse de Doctorat, Université de Liège (Belgique). 199p.

Jijakli M.H., 2007, «Synthèse bibliographique : problématique de la jacinthe d'eau, Eichhorniacrassipes, dans les régions tropicales et subtropicales du monde, notamment son éradication par la lutte biologique au moyen des phytopathogènes», Biotechnology, Agronomy, Society and Environment 11 : 299311.

McVea C., Boyd C.E. 1975, « Effects of water hyacinth cover on water chemistry, phytoplankton, and fish in ponds»,. Journal of Environmental Quality 4: 375378. In: EPPO European and mediterranean Plant Protection Organization 2008. Data sheets on quarantinepests. Fiches informatives sur les organismes de quarantaine Eichhorniacrassipes. Bulletin OEPP/EPPO 38: 441449.

Rodier J., 1984.L'analyse de l'eau :eauxnaturelles, eauxrésiduaires, eau de mer. 7è edition Bordas Paris. 1362 p.

Sankar Ganesh P., Ramasamy E.V., Gajalakshmi S., Abbasi S.A. 2005, « Extraction of volatile fatty acids (VFAs) from water hyacinth using inexpensive contraptions and the use of the VFAs as feed supplement in conventional biogas digesters with concomitant final disposal of water hyacinth as vermicompost», Biochemical Engineering Journal 27: 1723.

USEPA., 1988, «Design manual, constructed wetlands and aquatic plant systems for municipal wastewater treatment» United States Environmental Protection Agency, 83 pages.