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# **Wastewater treatment and resource recovery for poverty alleviation: a combined duckweed and water hyacinth pond system**

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Wastewater treatment and resource recovery under influence of fluctuating loading rate,  
heavy metal content, pH and light intensity and their effect on pollutants removal  
performance and resource recovery in ponds

Sena Peace HOUNKPE (B.Eng., MSc.)



A thesis submitted for the degree of Doctor of Philosophy

The University of Edinburgh

January 2016

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## ***Declaration***

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I declare that the research reported in this thesis has been conducted by myself (Sena Peace Hounkpe) and the work contained in it is my own, except where stated otherwise. Further, this thesis has not been submitted for the award of any other degree or professional qualification except as specified. Where other sources are quoted full references are given.

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January 28<sup>th</sup> 2016

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To my husband Enoc Edgad Wendeou

To my children Ruth-Doryane-Sarah-Berenice

*Success is down a long road strewn with various challenges which are overcome with a strong will, courage, perseverance and self- discipline.*

**Sena Peace HOUNKPE, 2015**

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## ***Abstract***

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Floating macrophyte pond systems, with the ability to produce nutrient enriched plants simultaneously with wastewater treatment, are a sustainable solution to contribute to environmental protection and safe nutrient recovery from domestic wastewater. However, to meet the requirements for reuse with high strength wastewater containing high levels of metal pollution generated in developing countries, an adequate combination of water hyacinth and duckweed ponds is proposed in order to take advantage of the best characteristics of each of these macrophyte ponds.

This research focused on the advancing of the understanding of the effectiveness of treatment and resource recovery under the effect of changing operational parameters such as pH, light intensity, influent metal content and fluctuating pollutants loading rate on pond performance and recycling ability in order to fill the noticed gap of knowledge.

Experiments conducted in water hyacinth ponds (WHP), under batch and tropical natural weather conditions, revealed that pH between 6.4 and 7.1, full sunlight and seven days hydraulic retention time were optimum for plant biomass production and pollutant removal in WHP. WHP was able to regulate pH when the initial pH values moved outside this interval with a drop in biomass production as a side effect. These ponds showed a first order kinetic for the removal of iron, zinc and copper from aqueous solution and their accumulation in plants biomass with a preferential sequence  $Fe > Zn > Cu$ . However the presence of metals in water hyacinth biomass led to the reduction in ponds performances and a risk of re-pollution of the effluent through the release of metals into water.

A comparative study carried out over sixty-two weeks in a pilot scale combined water hyacinth and duckweed ponds (DWP) channel and waste stabilization ponds channel working under fluctuating loading rates showed different

environmental conditions occurred these ponds. The fluctuating loading rate was also found to have a reduced effect on the combined WHP/DWP channel performance and effluent quality stability with the effluents meeting the entire reuse requirement at high hydraulic flow rate (retention time greater than 20 days). Fish was able to grow in the WHP/DWP channel.

Results suggested some guidelines on WHP/DWP system design, operation and maintenance. The overall outcome of this research is a significant contribution to the development of integrated combined WHP/DWP technology for treatment of wastewater and resource recovery on site.

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## **Résumé**

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Un système de bassins à macrophytes flottants, avec sa capacité de production de biomasse riche en éléments nutritifs tout en épurant les eaux usées domestiques, est une belle perspective pour la protection de l'environnement et le recyclage sans risque des ressources dans nos pays en voie de développement. Toutefois, avec la forte charge organique et en métaux lourds des eaux usées domestiques générées, pour obtenir le niveau de qualité requise pour la réutilisation des effluents, une combinaison adéquate des bassins à jacinthe d'eau (WHP) et à lentilles d'eau (DWP) est proposée afin de bénéficier des caractéristiques avantageuses de chacune de ces deux plantes.

Les présents travaux de recherche se sont focalisés sur l'amélioration du niveau des connaissances de la performance épuratoire et la capacité de production de ressources du système WHP/DWP soumis à différentes conditions de pH, d'intensité de lumière, de charge en polluants métalliques et de fluctuation des charges entrantes de polluants afin de combler le gap observé dans les littératures.

Des expériences conduites dans des bassins à jacinthes d'eau, alimentés en batch dans les conditions climatiques tropicales, ont révélé que pour une performance optimale d'épuration et une production optimale de biomasse, le pH soit entre 6,4 et 7,1; que le bassin reçoive directement les rayons solaires et ait un temps de séjour hydraulique moyen de sept jours. Toutefois, la jacinthe d'eau a montré une aptitude à réguler le pH à une valeur optimale lorsque le pH entrant est hors de cet intervalle avec comme corollaire une baisse de productivité. WHP a aussi montré son efficacité à éliminer des solutions aqueuses, suivant une équation cinétique de premier ordre le fer, le zinc et le cuivre et à les accumuler dans leur biomasse à la séquence préférentielle Fe>Zn>Cu. Toutefois la présence des métaux dans la biomasse de la jacinthe d'eau réduit ses capacités à épurer

les autres polluants et constitue un risque accru de repollution des effluents par le relargage des métaux.

L'étude comparative menée sur une durée de soixante-deux semaines, dans une station pilote grandeur nature entre une filière de système mixte de bassins à jacinthe d'eau et de bassins à lentilles d'eau (WHP/DWP) et une filière de bassins de stabilisation (WSP) alimentées par des charges entrantes fluctuantes, a montré que différentes conditions environnementales prévalaient dans les divers types bassins. Aussi, la fluctuation de la charge entrante se trouva avoir peu d'influence sur la performance et la stabilité de la qualité de l'effluent de la filière WHP/DWP avec des effluents respectant les normes de réutilisation à faibles charges hydrauliques (temps de séjour supérieur à 20 jours). Des expériences de pisciculture ont été faites avec succès en utilisant le dernier DWP comme bassin à poisson.

Ces résultats ont permis de faire des suggestions pour la conception et l'exploitation de systèmes mixtes WHP/DWP. Globalement, il ressort de ce travail de recherche une contribution significative au développement de la technologie du système intégré mixte de WHP/DWP pour le traitement et le recyclage des eaux usées domestiques.

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# Contents

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Declaration.....	ii
Thesis Supervisors .....	ii
Abstract.....	v
Résumé.....	vii
Acknowledgments.....	ix
Contents.....	xi
List of abbreviations .....	xvi
List of figures.....	xix
List of tables.....	xxi
<b>Chapter 1: Introduction .....</b>	<b>1</b>
1.1 Background and problem statement .....	1
1.2 Aim and objectives.....	7
1.3 Thesis outline .....	8
<b>Chapter 2: Literature Review .....</b>	<b>11</b>
2.1 Introduction.....	11
2.2 Waste Stabilization ponds.....	12
2.2.1. Definition and history .....	12
2.2.2. Classification of waste stabilization ponds.....	13
2.2.2.1 <i>Anaerobic ponds</i> .....	13
2.2.2.2 <i>Facultative ponds</i> .....	14
2.2.2.3 <i>Maturation ponds</i> .....	14
2.2.3. Pollutant removal mechanisms in waste stabilization ponds .....	14
2.2.3.1. <i>Organic matter removal</i> .....	14
2.2.3.2. <i>Nutrient removal (nitrogen and phosphorus)</i> .....	16
2.2.3.3. <i>Pathogen removal</i> .....	16
2.3 Macrophyte based ponds .....	17

2.3.1.	Importance of macrophyte growth in pollutant removal .....	17
2.3.2.	Macrophyte pond classification.....	18
2.3.3.	Water hyacinth and water hyacinth ponds.....	20
2.3.4.	Duckweed and duckweed ponds.....	24
2.4	Organic load, nutrient and pathogen removal mechanisms and recycling in macrophyte ponds .....	27
2.5	Metal removal mechanism in macrophyte ponds .....	29
2.5.1.	Metal toxicity and sources in wastewater .....	29
2.5.2.	Metal removal in water hyacinth ponds .....	31
2.6	Factors affecting plant growth and performance in macrophytes ponds	34
2.6.1.	Temperature .....	35
2.6.2.	pH.....	36
2.6.3.	Light intensity .....	36
2.6.4.	Conductivity and salinity .....	37
2.6.5.	Dissolved oxygen and plant coverage density .....	38
2.6.6.	Organic loads and nutrients.....	38
2.7	Macrophytes ponds and mosquito breeding .....	38
2.8	Concluding Discussion.....	39
<b><u>Chapter 3: Materials and methods.....</u></b>		<b>41</b>
3.1	Introduction.....	41
3.2	Study site description .....	42
3.3	Experimental set up .....	46
3.3.1.	Mini ponds.....	46
3.3.2.	Pilot plant.....	49
3.4	Operation.....	51
3.4.1.	Environmental conditions .....	51
3.4.2.	Mini-ponds operation .....	52
3.4.3.	Pilot plant operation.....	52
3.4.4.	Plant material .....	55

3.4.5.	Wastewater and culture medium .....	56
3.5	Risk assessment and safe system of work.....	57
3.6	Analytical methods .....	58
3.6.1.	Pilot plant inflow measurement .....	58
3.6.2.	Organic loading rate determination.....	58
3.6.3.	Plant growth measurement .....	59
3.6.4.	Environmental parameter measurement .....	59
3.6.5.	Suspended solids, organic matter and nutrients determination.....	60
3.6.6.	Microbial quality determination.....	62
3.6.7.	Metal pollutant determination.....	62
3.7	Results analysis method .....	63
3.7.1.	Analysis of variance: ANOVA.....	63
3.7.2.	Principal components analysis (PCA) .....	63
3.7.3.	Boxplots.....	65
<b><u>Chapter 4: Influence of solar radiation on water hyacinth ponds performance using high strength sewage</u></b> .....		<b>66</b>
4.1	Introduction.....	66
4.2	Materials and methods overview.....	67
4.3	Results and discussion.....	68
4.3.1.	Performance of anaerobic treatment .....	68
4.3.2.	Environmental parameters in water hyacinth ponds.....	69
4.3.3.	Pollutant removal performance .....	72
4.3.4.	Plant growth rate .....	74
4.3.5.	Discussion .....	76
4.4	Conclusions and recommendations.....	78
<b><u>Chapter 5: Influence of pH on water hyacinth ponds performance using medium strength sewage</u></b> .....		<b>79</b>
5.1	Introduction.....	79
5.2	Materials and methods overview.....	80
5.3	Results and discussion.....	81

5.3.1.	Performance of the anaerobic treatment .....	81
5.3.2.	pH and evolution of the environment parameters .....	82
5.3.3.	pH and evolution of pH in ponds .....	84
5.3.4.	pH and organic matter and nutrient removal .....	86
5.3.5.	pH and microbial pollution removal .....	91
5.3.6.	pH and plant biomass production .....	92
5.4	Conclusions .....	93
<b><u>Chapter 6: Heavy Metal Removal by water hyacinth: kinetic and performance</u></b>		
.....		<b>95</b>
6.1	Introduction.....	95
6.2	Materials and methods overview.....	96
6.3	Results and discussions .....	98
6.3.1.	Environmental parameters.....	98
6.3.2.	Kinetics of metal removal from solution.....	99
6.3.3.	Performance of metal removal from solution.....	103
6.3.4.	Metal accumulation in plants.....	104
6.3.5.	Plant growth .....	106
6.3.6.	Analysis of the relation between plant growth, metal removal and accumulation in biomass.....	108
6.4	Conclusions and recommendations.....	109
<b><u>Chapter 7: Effect of metal accumulation by water hyacinth on ponds performances</u></b>		
.....		<b>111</b>
7.1	Introduction.....	111
7.2	Materials and methods overview.....	113
7.3	Results and discussions .....	114
7.3.1.	Environmental parameters.....	114
7.3.2.	Metal in plants and performance of Ponds.....	115
7.3.3.	Metal in plants and their growth.....	118
7.3.4.	Release of metal by plants in water.....	119
7.4	Conclusions and recommendations.....	120

<b>Chapter 8: Environmental parameters in combined water hyacinth/duckweed treatment systems and algae based systems.....</b>	<b>122</b>
8.1 Introduction.....	122
8.2 Materials and methods overview.....	124
8.3 Results and discussion.....	125
8.4 Conclusions and recommendations.....	133
<b>Chapter 9: System performance variability under fluctuating loading rates in combined water hyacinth/Duckweed ponds and algae based ponds .....</b>	<b>135</b>
9.1 Introduction.....	135
9.2 Materials and methods overview.....	136
9.3 Results and discussions .....	137
9.3.1. Environmental parameters.....	137
9.3.2. Comparative effect of fluctuating loading rate on the combined WHP/DWP and WSP.....	138
9.3.3. Performance comparison of the combined WHP/DWP channel and WSP channel .....	149
9.3.4. Aquaculture and composting experiments.....	155
9.4 Conclusions and recommendations.....	156
<b>Chapter 10: General conclusions and design guidelines .....</b>	<b>159</b>
10.1 General conclusion.....	159
10.2 Design guidelines and recommendations .....	164
10.2.1. Hydraulic retention time .....	164
10.2.2. Organic loading rate.....	164
10.2.3. Faecal coliform die-off.....	165
10.2.4. Influent pH, light intensity and metal content .....	165
10.2.5. Fish culturing .....	166
10.2.6. Construction and protective measures .....	166
10.2.7. Operation and maintenance.....	167
10.3 Recommendation for future research.....	167
<b>References .....</b>	<b>169</b>

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## ***List of abbreviations***

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### **General**

AFNOR	Association Française de NORmalisation (French Association for Normalisation)
ANOVA	Analysis of variance
ASECNA	Agence pour la Sécurité de la Navigation Aérienne en Afrique et Madagascar
COUS AC	Centre des Œuvres Universitaires et Social d'Abomey Calavi (University of Abomey-Calavi Social and Help Center)
DWP	Duckweed Pond
EPAC	Ecole Polytechnique d'Abomey-Calavi (Polytechnic School of Abomey-Calavi)
INSAE	Institut National de Statistique et d'Analyse Economique (National Institute of Statistics and Economic Analysis)
LCQEA	Laboratoire de Contrôle de Qualité des Eaux et Aliments (Laboratory of Water and Food Quality Control)
LSTE	Laboratoire des Sciences et Techniques de l'Eau (Laboratory of Water Science and Techniques)
MP	Mini-Pond
PCA	Principal Component Analysis
RGPH	Recensement Général des Personnes et des Habitats (General Census of Population and Housing)
RGR	Relative Growth Rate
SONEB	Société Béninoise d'Electricité et d'Eau (Benin Water Company)
WHO (or OMS)	World Health Organisation (Organisation Mondiale de la Santé)

WSP	Waste Stabilization Pond
WHP	Water hyacinth Pond

### Parameters

BOD <sub>5</sub>	Five-day at 20 °C Biochemical Oxygen Demand (mg/l)
COD	Chemical Oxygen Demand (mg/l)
DO	Dissolved oxygen (mg/l)
e <sub>H</sub>	Redox Potential
FC	Faecal Coliforms (number/100 ml or number/l)
FW	Fresh weight (mg)
HFR	Hydraulic flow rate
HLR	Hydraulic loading rate
λ <sub>v</sub>	Volumetric loading rate (mg/m <sup>3</sup> )
pH	Potential of hydrogen
Pt	Total Phosphorus (mg/l)
Q	Flow rate (m <sup>3</sup> /day)
T	Temperature (°C)
t	Time
TDS	Total Dissolved Solids (mg/l)
TN	Total Nitrogen (mg/l)
TKN	Total Kjeldhal Nitrogen (mg/l)
TP	Total phosphorus (mg/l)
TSS	Total Suspended Solids (mg/l)
Turb	Turbidity (NTU)
χ	Electrical Conductivity (μS/cm)

### Units

g	Gram
ha	Hectare
kg	Kilogram

m	Metre
mg/l	Milligrams per litre
NTU	Nephelometric Turbidity Units
s	Second
cm	Centimetre
$\mu\text{S}$	Micro-Siemens
$\mu\text{E}$	Micro-Einsteins

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## **List of figures**

---

Figure 2. 1 : Symbiotic relationship between algae and bacteria in stabilization ponds (adapted of (Feachem and Cairncross 1978)) .....	15
Figure 2. 2 : Schematic representation of different types of macrophyte pond (Brix, 1991, Vymazal, 2005) .....	18
Figure 2. 3 : Water hyacinth (Houngkpè 2013) .....	21
Figure 2. 4 : Duckweed (Wendeou et al. 2013) .....	24
Figure 3. 1 : Map of Study area (adapted from(Bénin), 2013).....	42
Figure 3. 2 : Pilot plant location on the University campus (adapted from (Bénin ; Cruz et al. 2013)).....	45
Figure 3. 3 : Layout of the experimental set up with mini-ponds .....	47
Figure 3. 4 : A view of the experimental set up with mini-ponds .....	47
Figure 3. 5 : Experimental set-up with mini-ponds with artificial medium as influent. ....	48
Figure 3. 6 : General arrangement of each channel of the system .....	50
Figure 3. 7 : Pilot Plant Set-up .....	51
Figure 3. 8 : Total flow rate of the system over the experimental period.....	53
Figure 3. 9 : Sampling points in the system.....	54
Figure 3. 10 : Sampling for organic load, nutrient and faecal coliforms content.....	54
Figure 4. 1 : Changes in light intensity in ponds.....	69
Figure 4. 2 : Evolution of the temperature .....	69
Figure 4. 3 : Evolution of the pH .....	70
Figure 4. 4 : Evolution of the Conductivity .....	71
Figure 4. 5 : Evolution of the turbidity .....	72
Figure 4. 6 : Organic matter and nutrients removal rate from water.....	73
Figure 4. 7 : Some ponds at the end of the experimental period.....	75
Figure 4. 8 : Change in relative growth rate to plants number.....	76
Figure 5. 1 : Evolution of temperature in ponds .....	82
Figure 5. 2 : Evolution of electrical conductivity in ponds .....	83
Figure 5. 3 : Evolution of electrical conductivity in ponds .....	84

Figure 5. 4 : Evolution of pH in ponds .....	85
Figure 5. 5 : Performance changes of water hyacinth with influent at different pH.....	87
Figure 5. 6 : Plant relative growth related to the masse and number of plants as function of influent pH .....	92
Figure 6. 1 : Experimental ponds at the beginning of the experiment .....	97
Figure 6. 2 : Metal removal kinetics models for Cu, Fe and Zn removal by water hyacinth.....	101
Figure 6. 3 : Percentage of metals removal from water .....	103
Figure 6. 4: Metal concentration in biomass as function of initial concentration of metal in solution.....	105
Figure 6. 5 : Relative growth rate of water hyacinth as function of type of metal and its concentration in solution.....	106
Figure 6. 6 : Projection of the variables into the factorial plan (F1, F2) .....	109
Figure 7. 1 : COD, TSS, TNK and phosphate removal rates as function metal in plants .....	115
Figure 7. 2 : Water hyacinth relative growth rate as function of plant source .....	118
Figure 8. 1 : PCA correlation circle.....	126
Figure 8. 2 : Changes in pH, $e_H$ and temperature along the treatment channels.....	125
Figure 8. 3: Changes in TDS and conductivity along the treatment channels.....	130
Figure 8. 4 : Changes in environmental conditions of the influent and effluent of ponds .....	132
Figure 9. 1: Influent and effluent TSS, COD, TN and phosphate as function of HFR	141
Figure 9. 2: Microbial quality of the influent and the effluent as function of hydraulic flow rate.....	144
Figure 9. 3 : COD removed from each channel as function of influent COD.....	147
Figure 9. 4: DO, COD, BOD <sub>5</sub> , TSS removal rates evolution along the two channel...	151
Figure 9. 5 : Phosphate, Nitrate, TN and faecal coliform removal in WHP/DWP channel and WSP channel.....	153
Figure 9. 6 : Fish caught from the duckweed fish pond .....	156

---

## **List of tables**

---

<i>Table 3. 1 : Greywater, wastewater and septage characteristics of Cotonou and Abomey-Calavi University adapted from (Hounkpè et al. 2013) .....</i>	44
<i>Table 3. 2 : Pilot Plant Pond dimensions .....</i>	49
<i>Table 3. 3 : Typical composition of wastewater with different strength .....</i>	56
<i>Table 3. 4 : Composition of Hillman solution prepared for the experiment (Landolt, 1987).....</i>	57
<i>Table 4. 1 : Raw wastewater and anaerobic pond effluent .....</i>	68
<i>Table 5. 1 : Raw wastewater and anaerobic pond effluent .....</i>	81
<i>Table 5. 2 : p-values of removal performances in relation to pH.....</i>	88
<i>Table 5. 3 : Coliform count in influent and effluent of ponds .....</i>	91
<i>Table 6. 1 : Kinetic parameters for Cu, Fe and Zn removal by water hyacinth .....</i>	100
<i>Table 6. 2 : Correlation matrices between variables and factors .....</i>	108
<i>Table 7. 1 : Characteristics of the previous culture medium and metal concentration in plants material.....</i>	114
<i>Table 7. 2 : Metal release rate by water hyacinth in water .....</i>	120
<i>Table 8. 1 : Correlation matrix (Pearson (n)).....</i>	127
<i>Table 9. 1 : Channels loading rate and removal rate of TSS, COD and TN over the experimental period .....</i>	139
<i>Table 9. 2: Channels loading rate and removal rate of phosphate and faecal coliforms over the experimental period.....</i>	140
<i>Table 9. 3: Variance of the effluent quality parameters of WHP/DWP and WSP channels .....</i>	143
<i>Table 9. 4: First order faecal coliforms die-off values for the two channels.....</i>	146

# 1

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## ***Introduction***

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### **1.1 Background and problem statement**

The destruction of the ozone layer, climate change, water shortages and epidemics are among so many disasters that affect humanity in recent times, putting environmental protection and public health at the heart of the concerns of nations and international organizations, and nowadays giving them a prominent place in development policies. This situation puts wastewater management as the highest of priorities because wastewater is one of the main sources of diseases and pollution of waters and soil. Indeed, according to the WHO, most of all illnesses in developing countries are caused by water and sanitation related diseases (Franceys *et al.* 1995; WHO. *et al.* 2000; WHO and UNAIDS. 2004). For instance diarrhoea, a waterborne disease, is in second place of infections contributing to the global burden of disease – ahead of heart disease and human immunodeficiency virus (HIV) / acquired immunodeficiency syndrome (AIDS) (WHO 2010).

The need for sanitary facility installations is essential as it prevents contamination of water and soil by human faecal matter and epidemics of waterborne diseases (WHO. *et al.* 2000). This need is particularly important in urban areas of developing countries, such as Cotonou, Benin, where the volume of wastewater to manage is becoming increasingly important as a result of high population growth, for instance 3.5% of growth rate for Benin (Mbéguééré, *et al.*, 2010; Insae 2013).

Sanitation in the West Africa's major cities is characterized by individual sanitation facilities which are not standardized, and generally in direct contact with soil or groundwater. These facilities are made to collect mainly excreta; grey water, from bathrooms; kitchen, laundry or use for other purposes, is discharged directly into the environment. The septage from individual sanitation facilities is discharged into the environment or is directed to treatment plants where it receives in most cases very poor treatment with plant effluent failing to meet any discharge guidelines (Collignon 2002; Bolomey *et al.* 2003; SEIDL *et al.* 2003; Koanda 2006; Hounkpè *et al.* 2014)

For example in Cotonou more than 80% of grey water is discharged directly onto the streets, in the public storm drains or in courtyards. More than 91% of the excreta are collected by shallow individual sanitation facilities while about 6% of them are discharged into the environment through open defecation (Hounkpè *et al.* 2014). This situation is a source of high pollution of water wells which can contain up to 292 000 coliforms/l (Hounkpè, 2002). This water from wells is used by more than 81% of the population for domestic purposes and by about 5% of the population as drinking water. The septage from individual facilities is treated in the only existing wastewater treatment plant of Ekpe, which is supposed to treat 180m<sup>3</sup> of wastewater a day but is now receiving more than 450m<sup>3</sup>, with a large variation of the loading rate throughout the week. This has led to a deterioration of effluent quality with BOD<sub>5</sub> removal as low as 5% (Yadouleton 2002; Hounkpè *et al.* 2014).

This domestic wastewater, which contains faeces and is a source of all sorts of pollution, also contains valuable substances which can be used as a source of plant nutrients. But, the current treatment facilities available in Cotonou (Benin), as they are performing at present, are inadequate to demonstrate any usefulness of this wastewater to the public as there is no attempt to recover nutrients in order to reduce treatment cost. It has, however, been reported that municipal wastewater treatment cost can be reduced substantially by applying cost-effective treatment technologies and using treated water for crops and fish

farming that could generate processing costs (Faruqui *et al.* 2003; Akponikpè *et al.* 2011).

Another shortcoming of the wastewater management system in developing urban areas like Cotonou is that the treatment system is centralized, whilst the collection at household level is individual with no application of central authority or even participation by the local community as a whole. Also the collection and transport of the wastewater to treatment plant is managed by private companies without the involvement of the community (Houunkpè *et al.* 2014). This situation affects the efficiency of the system and contributes to increasing environmental pollution problems.

The solution to these problems is to adopt an adequate management approach with an improved technology for wastewater treatment suitable for our environment that could be easy to operate and allow cost recovery. A general awareness about the depletion of natural resources, the need to promote the integrated water resources management and the inadequacy of the conventional sanitation systems (Mara 1996; Koné *et al.* 2002; Tsagarakis *et al.* 2003; Mara 2004; Awuah 2006; Oliveira and Von Sperling 2008; Konnerup *et al.* 2009) calls for the use of macrophyte ponds, an environmental friendly, less energy-intensive and cost-effective system, to treat and recycle nutrients from wastewater.

Macrophyte ponds, also called “green wastewater treatment”, are modified waste stabilization ponds (WSP) in which aquatic plants are used to improve effluent quality. Macrophyte ponds are designed to imitate physical, chemical and biological processes found in natural wetland ecosystems to remove contaminants from the wastewater. They rely on natural ecological processes, thus are a better alternative to the more energy and chemical intensive "conventional" systems (Rodriguez and Jensen 2005; Nasr *et al.* 2008).

Several aquatic plants have been tested for wastewater treatment. All these plants, including floating (water hyacinth, water lettuce, duckweed), submerged (algae, *Lemna trisulca*), or emergent (*Cyperus papyrus*, bulrush, reed) have shown

their efficiency in removing pollutants from water (Brix 1997). The most used floating aquatic plants in wastewater treatment are water hyacinth, water lettuce and duckweed and several treatment plants using them exist in the world (Ho and Wong 1994; Perdomo *et al.* 1999; Al-Nozaily 2001; Caicedo Bejarano 2005; Awuah 2006; Zimmels *et al.* 2006; Alvarado *et al.* 2008; Zheng *et al.* 2013).

Water hyacinths have proved, through several laboratory as well as pilot scale and full scale studies, their efficiency and are better than WSP in removing and recycling organic pollutants and nutrients from wastewater. These plants have high nutrient up-take capacity and can grow under strong wastewater (Reddy *et al.* 1982; Brix 1994; Maharjan and Ming 2012). Due to their ability of growing under completely nutrient-poor conditions and their suitable morphology, water hyacinths are tested and reported as up-taking efficiently different toxic metals from contaminated wastewater (Cordes *et al.* 2000; Jayaweera *et al.* 2007; Mane *et al.* 2011; Gakwavu *et al.* 2012; Saleh 2012; Smolyakov 2012). Nevertheless, water hyacinth ponds have shown their limits in pathogen removal and it has been reported that the effluent from these ponds requires polishing before discharge or reuse (Polprasert *et al.* 1992; Adewunmi *et al.* 2009; Maharjan and Ming 2012). Also, the acidic quality of the effluent reported by several literatures (Kim and Kim 2000) casts a doubt in their reuse in agriculture.

Duckweed, on the other hand, has been reported very efficient in nutrient and pathogen removal. Faecal coliform die-off of 3 to 4 log unit in duckweed ponds treating domestic wastewater were reported (Alaerts *et al.* 1996; Awuah *et al.* 2004; Caicedo Bejarano 2005; Nasr *et al.* 2008). The effluent from duckweed based treatment systems, with up to 6mg/l of DO and pH around neutral values, met the requirement for reuse or discharge (Al-Nozaily 2001; Caicedo Bejarano 2005; Awuah 2006). On the other hand, these plants cannot withstand high BOD<sub>5</sub> or nitrogen concentrations over 100 mg/l in the influent, which makes duckweed ponds difficult to use for strong wastewater treatment (Zimmo *et al.* 2005).

Experience has shown that no single technology can offer an optimum treatment for the different components to be removed from wastewater, including organic matter, suspended solids, metals, nutrients and pathogens, or allow for valuable resource recovery. Suitable combinations of water hyacinth with duckweed in a system may produce superior organic loads, nutrient and pathogen removal compared with water hyacinth or duckweed alone. Putting water hyacinth ponds as a secondary treatment will reduce the organic and metal loads on duckweed ponds; duckweed ponds as tertiary treatment will increase the system performance in pathogens removal and the possibility of reuse of the effluent.

An integrated macrophyte pond system is therefore proposed combining two different macrophyte ponds in order to take advantage on the best characteristics of each macrophyte.

Such a treatment system, if locally managed and integrated with resource recovery such as fish farming and urban agriculture may help in solving wastewater management problems in developing urban cities such as Cotonou. It will promote rational use of resources and can allow for cost recovery. The use of participatory approach in respect of the community in the management of the system will increase the community involvement and the sustainability of the project.

On the other hand, highly fluctuating wastewater quality with high metal content due to the pollution of wastewater by artisanal industries and the disposal of materials such as oil spills, batteries and detergents in wastewater streams was reported in developing urban areas. An analysis carried out on domestic wastewater characteristics of the city of Cotonou, for example, showed it may contain Fe, Cu and Zn up to 87 mg/L, 45 mg/L, 23 mg/L, respectively (Hounkpè *et al.* 2013).

It is also necessary, before using the proposed system, to have an understanding on the effect of changing parameters such as pH, light intensity and metal

content on the pond performance and the macrophyte biomass production, especially water hyacinths, which are receiving anaerobically treated wastewater and are thus more exposed to these changes and high pollutant concentrations. Also, the effect of the fluctuating organic loading on the system should be assessed for better design and management.

Due to the on the gap in the understanding of which parameters which affect the efficiency of such a combined macrophyte system, the present research work aims to develop understanding of the design, operation and maintenance of the system in developing tropical communities facing fluctuating influent wastewater quality. The effect of other parameters such as pH, light intensity and metal pollution on ponds efficiency will also be studied.

The method of research is based on experimental studies carried out in short incubation batch scale mini-ponds and a continuous flow pilot plant installed on the Campus of the Université d'Abomey-Calavi (UAC) of Benin. In designing and operating the experimental systems to solve the identified problems the research sought to answer specific questions which surfaced such as:

- Q1) What is the effect of light intensity on macrophyte pond performance and biomass production for resources recovery?
- Q2) How and to what extent does influent pH variability (acidic, neutral, and alkaline) affect treatment efficiency and biomass production for resources recovery?
- Q3) How effective are macrophyte ponds in removing major metals found in domestic wastewater in developing cities (iron, zinc and copper) from aqueous solution?
- Q4) What is the mechanism of metal removal in macrophyte ponds?
- Q5) How does the presence of these metals in the water affect biomass production?
- Q6) How do the quantity and type of metals previously accumulated in macrophytes affect treatment efficacy and biomass production?

- Q7) How is the efficiency of macrophyte ponds affected by the metal accumulated in plants?
- Q8) Is there any risk of re-pollution of the effluent through release of metal by plants?
- Q9) What are the environmental conditions and their evolution in the combined water hyacinth ponds and duckweed ponds (WHP/DWP) system?
- Q10) How efficient is the combined WHP/DWP system compared to WSP system?
- Q11) How and to what extent do fluctuating hydraulic and organic loading rates influence treatment efficiency in the combined WHP/DWP system?
- Q12) Is the effluent quality of combined WHP/DWP system more stable under fluctuating loading rates compared with WSP system and can it be easily reused in fish farming and agriculture?

The intensive monitoring data and research results which this study seeks to provide will be useful in establishing a solution to the above mentioned problems and in managing combined WHP/DWP system for wastewater treatment and resource recovery treatment opportunities for future.

## **1.2 Aim and objectives**

Physico-chemical conditions affect both plant growth and biological processes in an integrated macrophyte pond system and as a consequence its performance in terms of removal efficiencies and biomass production. The overall aim is therefore to advance understanding of the application of an integrated WHP/DWP system for wastewater treatment and resource recovery.

In order to identify and quantify the fundamental mechanisms involved in the pollutants removal in an integrated WHP/DWP system and the condition affecting performances in ponds, the specific objectives this thesis are to assess:

- 1- the role of solar radiation on water hyacinth pond (WHP) performance and biomass production (Q1);
- 2- the effect of influent pH variability on WHP efficiency and biomass production (Q2);
- 3- the performance, the process and the kinetic of heavy metals (Fe, Cu, Zn) removal in WHP and the effect of the presence on metals on biomass production (Q3, Q4 and Q5);
- 4- the effect of metal accumulation by water hyacinth on ponds performance, biomass production and the risk of re-pollution of effluent through metal release by plants (Q6, Q7 and Q8);
- 5- the environmental conditions in an integrated WHP/DWP system and compare their evolution in this system to that of a WSP system (Q9);
- 6- the effect of fluctuating hydraulic and organic loading rates on treatment efficiency of an integrated WHP/DWP, and to compare it with the performance, effluent stability and potential for sustainable reuse in fish culturing with that of a WSP system (Q10, Q11 and Q12).

### **1.3 Thesis outline**

The work described in this thesis started by reviewing of the existing information on macrophyte ponds for wastewater treatment and recycling. Then, in order to determine the optimal conditions for our systems, various tests were carried out. The results of the investigations and the experiments are reported in subsequent chapters as follows:

- Chapter 1 (this chapter) describes the background, statement of the problem, aim and objectives of the study together with this thesis outline;
- Chapter 2 presents the review of literature on macrophyte pond systems for wastewater treatment and recycling. An overview on waste stabilization ponds in general, and macrophyte ponds (water hyacinth and duckweed) in particular, enumerating the type of ponds and the factors affecting performance and plants growth is reported. A significant

proportion of the chapter is devoted to the published literature on the removal mechanisms of pollutants in ponds.

- Chapter 3 includes a detailed description of the study site, the experimental set-up (type of mini-ponds and pilot plant) and operation methods applied for the study. This chapter explains the analytical equipment and chemicals used to perform experiments and the results analysis methods. The chapter also documents the risk assessment done prior to the study and the security measures during operation.
- In chapter 4, the effect of solar radiation solar radiation on water hyacinth growth and pond performance using high strength wastewater ( $BOD_5 \approx 400 \text{ mg O}_2/\text{l}$ ) is reported.
- In chapter 5, the effect of the variability of the influent pH on water hyacinth pond performance and biomass production is examined with a medium strength wastewater ( $BOD_5 \approx 220 \text{ mg O}_2/\text{l}$ ).
- Chapter 6 looks at the performance and the process of heavy metal (iron, copper, zinc) removal in water hyacinth ponds on a batch scale and the effect of metals in water on plant biomass production using an artificial culture medium polluted by metals.
- The effect of previous exposure to heavy metal on water hyacinth removal performance of organic and nutrient loads and faecal bacteria are examined in Chapter 7, using anaerobically treated sewage. The biomass production and the possible related risk in reusing effluent in agriculture and fish farming are assessed.
- Chapter 8 reports on the comparison between environmental parameters prevailing in an integrated WHP/DWP system and an algae-based system (WSP) and their evolution as functions of changes in influent quality using small-scale pilot systems.

- Chapter 9 reports on the comparison between performance in pollutant removal and the stability of the effluent quality between an integrated WHP/DWP system and an algae-based system (WSP) under realistic fluctuating loading rates as might be obtained from a non-sewered waste water collection regime. The efficiency in nutrient and organic load removal and also the influence of fish in the last treatment pond on effluent quality are considered in this chapter.
- Finally, the general conclusions, the main recommendations on design, operation, and the maintenance requirements of the proposed integrated macrophyte-based treatment systems and the further research proposed are presented in Chapter 10.

# 2

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## **Literature Review**

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### **2.1 Introduction**

The era of escalating environmental crisis, rapid population growth and several compelling reasons have put the focus of much interest on the use of macrophytes to treat and recycle wastewater, especially in developing countries (Mbéguééré, *et al*, 2010; Insaé 2013).

This chapter summarises the scientific literature relating to pollutant removal and resource recovery in water treatment ponds. Particular focus is given to water hyacinth ponds and duckweed ponds which are natural and environmentally friendly systems enhancing efficiency and resource recovery through the presence of the macrophytes.

The first part of this chapter presents in-depth historical and technical review of existing information about waste stabilization ponds (WSPs) in general, showing the types and the removal mechanisms of contaminants in them. The scientific background of macrophyte ponds in general, and in particular, the characteristics of water hyacinth ponds (WHP) and duckweed ponds (DWP) are given in the second part. The next section describes the pollutant removal mechanism and resource recovery means in these macrophyte ponds with the subsections dealing with various removal processes. This is followed by a discussion of factors affecting efficiency in WHPs. Emphasis on metal removal mechanisms and reported performances is presented in the last part.

## 2.2 Waste Stabilization ponds

### 2.2.1. Definition and history

WSPs are alga-bacterial reactors which consist of a large, shallow man-made basin in which wastewater is retained long enough for natural, physical, chemical and biological purification processes to provide the necessary degree of treatment (Mara *et al.* 1992; Awuah 2006). The technology includes a pond or a system of ponds exposed to the air, which simulates and amplifies the self-purification action of lakes. At least part of the system must be aerobic to produce an acceptable effluent quality (Peavy Howard *et al.* 1985; Edeline 1993; Metcalf and Eddy 1995).

The first recorded construction of a WSP was in the USA at San Antonio, Texas, in 1901. This pond known, as Mitchell Lake, is an impoundment of 275 ha with an average depth of about 1.4 m, which is still in use (Gloyna 1972; Gloyna 1976; Reed *et al.* 1995). The first use of a pond system specifically designed to treat raw wastewater occurred in North Dakota and received the unconditional approval of the State Health Department in 1943. Following field work in 1940-1950, the development of rational design criteria for pond systems was undertaken and field research data began to appear after 1950 (Gloyna 1972). WSPs have undergone sufficient study and development to be classified as one of the major types of wastewater treatment systems.

WSPs have been introduced in West and the Central African trough with French and Swiss aid. The first ponds were installed in Senegal around 1976, between 1985 and 1987 in Burkina Faso, Cameroun and Cote d'Ivoire (Koné *et al.* 2002; Koanda 2006). Currently Waste Stabilization ponds are extensively used in many other countries such as Ghana, Nigeria and Benin (Gloyna 1972; Mara *et al.* 1992; Hounkpè *et al.* 2014).

WSPs require little or no mechanical equipment and thus relatively low capital investment cost when flat land is available at a reasonable price. They are low-cost processes for treating sewage, simple to build, reliable and easy to maintain.

Properly designed and operated pond systems provide pathogen removal which is better than conventional treatment processes and the effluent is often used for irrigation because of the nutrients it contains. The algae produced are a potential source of high-protein food, which can conveniently be exploited in fish farming. The main disadvantage of ponds is their large area requirement (WHO 1989; Mara *et al.* 1992; Pescod 1992; von Sperling 1996; Veenstra *et al.* 1997; SEIDL *et al.* 2003; Pedrero *et al.* 2010).

### **2.2.2. Classification of waste stabilization ponds**

WSPs can be classified according to their regime (anaerobic, facultative or aerobic) or to their position in the treatment line (primary, secondary, maturation or fish pond) (Edeline 1993).

#### **2.2.2.1 Anaerobic ponds**

The anaerobic ponds, with depth from 2 to 5m, are anaerobic digesters receiving a high BOD loading rate (over 100 mg/m<sup>3</sup>/d). The actual loading will depend on the climate, with higher rates at higher temperatures (Edeline 1993; Metcalf and Eddy 1995). The primary function of these ponds, in which oxygen is absent except for a relatively thin surface layer, is to remove organic matter (Effebi 2009).

The inconvenience of the anaerobic treatment is the possible production of odour which can be controlled by applying a BOD loading rate with respect to design guidelines and a sulphate loading rate less than 500 mg de SO<sub>4</sub><sup>2-</sup> /L. However the presence of sulphate in ponds is benefit as their reduction by bacteria (*Desulfovibrio*) in hydrogen sulphide allows for the precipitation of part of the metals present in the water and then removal by settling (Gloyna 1972; Mara *et al.* 1992).

### **2.2.2.2 Facultative ponds**

Facultative ponds are ponds in which aerobic conditions are maintained in the upper portions by oxygen generated by algae and, to a lesser extent by penetration of atmospheric oxygen, and anaerobic conditions prevail along the bottom due to stagnant conditions in the sludge, preventing oxygen transfer to that region (Gloyna 1972; Hammer 1991; Viessman and Hammer 2005). Facultative ponds, with depths varying from 1.5 to 2.5 m and retention time from 5 to 30 days, have as their main function BOD<sub>5</sub> and pathogen removal under a low volumetric loading rate (between 100 and 400 kg / ha.day of BOD<sub>5</sub>), to allow plants growth in the pond (Mara *et al.* 1992). Under favourable conditions, facultative ponds may be used as the total treatment system for municipal wastewater (Peavy Howard *et al.* 1985; Metcalf and Eddy 2003).

### **2.2.2.3 Maturation ponds**

Aerobic or maturation ponds are shallow ponds (1 to 1.5m deep), with very low biological and bacteriological vertical stratification, in which dissolved oxygen is present at all depths. They are most frequently used as additional processes to upgrade the effluent from a facultative pond or another maturation pond. These ponds are mainly intended for pathogen and suspended solids removal. Their number and the size depend on the bacteriological quality that is required of the effluent. They are not designed for BOD removal, yet their BOD removal is estimated at more than 25% (Gloyna 1972; Mara *et al.* 1992).

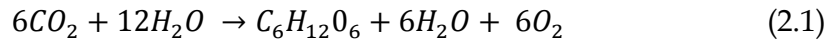
## **2.2.3. Pollutant removal mechanisms in waste stabilization ponds**

### **2.2.3.1. Organic matter removal**

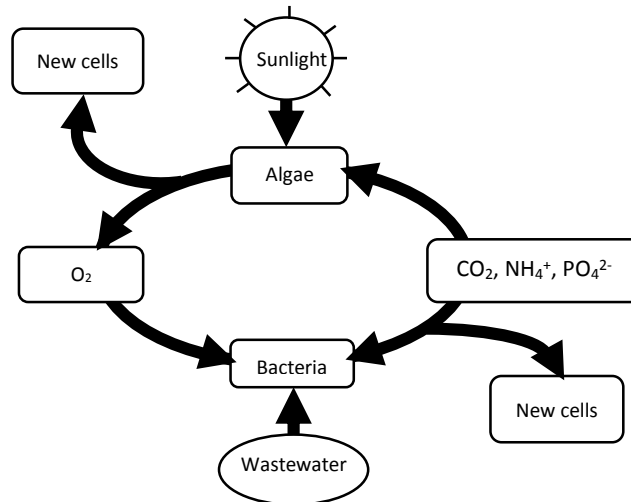
Organic matter is removed in anaerobic WSPs by sedimentation and subsequent anaerobic digestion. BOD will then leave the pond as methane; up to 80% but normally about 60% of BOD removal is possible under tropical climate conditions (Gloyna 1972; Mara *et al.* 1992; Edeline 1993). Indeed, Effebi has

reported that, under the tropical conditions, a mean annual removal of 72.21% of suspended solids, 70.86% of COD and 87.43% of BOD<sub>5</sub> (Effebi 2009).

In facultative ponds, the non-settlable BOD present is oxidised by the heterotrophic bacteria in the wastewater. The oxygen necessary for bacteria to digest the organic matter during day time is provided through the photosynthesis of algae under sunlight and is expressed by the following reaction (Gloyna 1972; Edeline 1993; Metcalf and Eddy 1995):



There is then a mutual relationship between the algae and bacteria in ponds during the day time (Figure 2.1). Thus most of the BOD which does not escape from the system as methane through anaerobic digestion will leave as algal biomass. It is for this reason that the effluent from WSP normally carries high amounts of suspended algae, which contributes to the BOD load and TSS content of the effluent and then poses a problem to receiving water bodies and in agricultural irrigation schemes. Its reuse for fish culturing can reduce this negative impact (Mara *et al.* 1992)



**Figure 2. 1 :** Symbiotic relationship between algae and bacteria in stabilization ponds (adapted of (Feachem and Cairncross 1978))

### **2.2.3.2. Nutrient removal (nitrogen and phosphorus)**

Organic nitrogen is hydrolyzed to ammonia in anaerobic ponds, in such a way that the concentration of ammoniacal nitrogen in the effluent of these ponds is generally higher than in the influent (Mara *et al.* 1992; Effebi 2009).

In facultative and maturation ponds, all the ammonia produced in the anaerobic pond is incorporated into new algal biomass, but algae are also able to assimilate free amino acids. When these algae die, they sink to the bottom of the ponds, and the non-biodegradable fraction (about 20%) of the incorporated nitrogen remains trapped in sediments and removed during desludging. The biodegradable fraction is recycled to the liquid in new algal cells. At high pH, nitrogen volatilization will occur. Very little nitrification, and then denitrification occurs in WSPs because they contain very low quantities of nitrifying bacteria (Mara *et al.* 1992; Edeline 1993; Caicedo Bejarano 2005; Camargo Valero and Mara 2007).

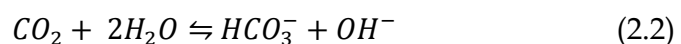
Phosphorus is removed from ponds by sedimentation and subsequent incorporation to algal biomass (Mara *et al.* 1992; Caicedo Bejarano 2005).

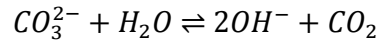
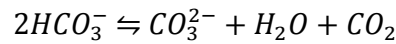
### **2.2.3.3. Pathogen removal**

In anaerobic ponds, a fair degree (70 to 95%) of helminth and nematode egg reduction can be achieved simply by providing a settling opportunity (Mara *et al.* 1992; Awuah 2006).

The main factors responsible for the die-off of pathogens in ponds are:

- Retention time and the temperature: the faecal bacteria die-off increases with the retention time and temperature (Metcalf and Eddy 1995);
- High pH (>9): the photosynthesis activities of algae consume the CO<sub>2</sub> faster than bacteria can produce it through respiration and this results in the decomposition of the carbonate and bicarbonate ions and then in the increase of the pH to above 10:





Faecal bacteria (apart from *Vibrio choléra*) quickly die, in less than one minute, at a pH>9 (Pearson *et al.* 1987; Henze *et al.* 2001);

- High light intensity from 400 to 700 nm wave length absorbed by the humic substances ubiquitous in wastewater puts the fecal bacteria in a state of excitation sufficient to damage their cells. This mechanism depends on the light intensity and is enhanced by a high pH (Curtis *et al.* 1992; Mara *et al.* 1992).

## 2.3 Macrophyte based ponds

### 2.3.1. Importance of macrophyte growth in pollutant removal

Macrophyte ponds, also called “green wastewater treatment”, are modified WSPs in which aquatic plants are used to improve the effluent quality (Rodriguez and Jenssen 2005; Nasr *et al.* 2008). As WSPs, they require a large surface area of land.

However, in tropical climates, macrophytes can make use of the favourable sunshine for their rapid growth and consequently fix a lot of soluble pollutants, especially nutrients, by converting them into new biomass. It has been proved that the presence of plants in a system makes it more efficient in decreasing suspended and settleable solids, BOD<sub>5</sub>, COD, nitrogen and phosphorus than conventional WSPs (EPA 1993; Kivaisi 2001; Rodriguez and Jenssen 2005; Sajin *et al.* 2005).

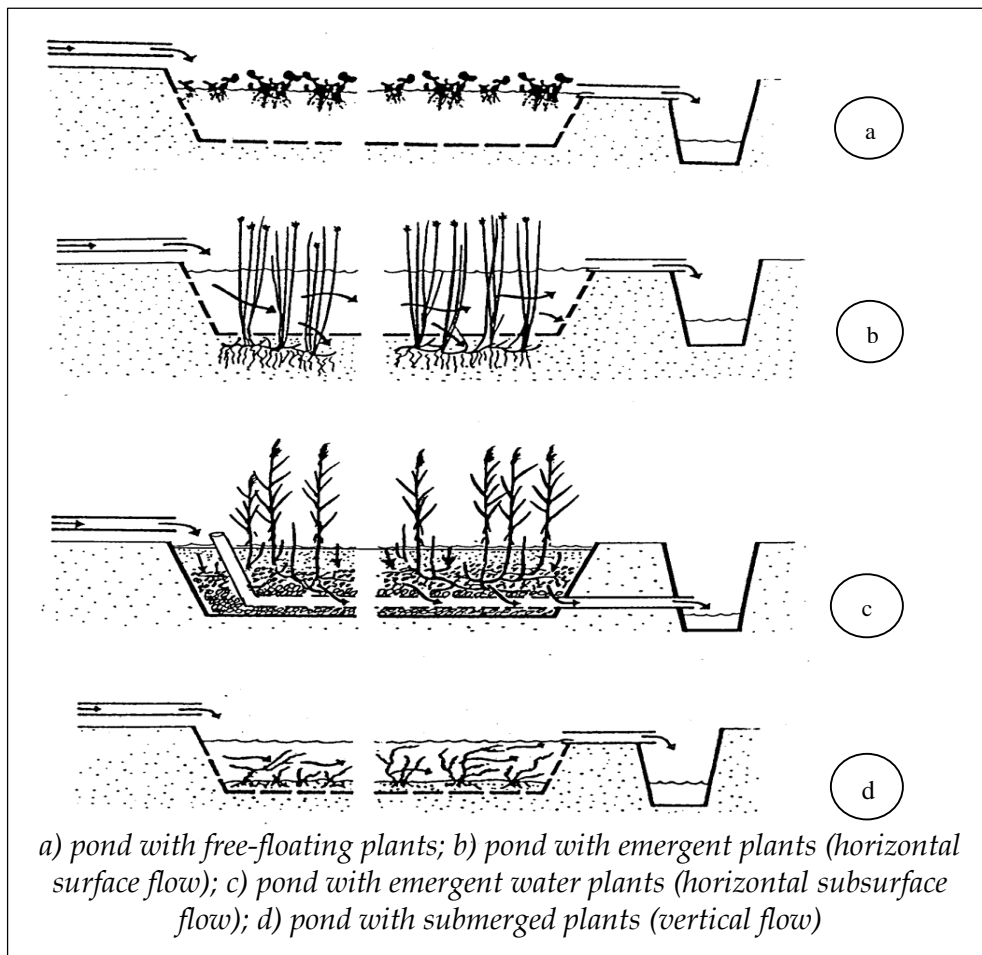
The purification effect is mainly due to the ability of plants to extract nutrients from the water column, the surface and the environment that they provide to the growth of aerobic bacteria able to degrade organic matter and nitrify ammonia nitrogen. Some macrophytes release sufficient oxygen in water for microbial activity (Kengne *et al.* 2000; Vymazal 2005).

Nevertheless, the relative contribution of plant pond water oxygen remains controversial. Some macrophyte pond designers assume that plant oxygen transport is significant (DeBusk *et al.* 2001), while others consider it as negligible (EPA 1988).

Another advantage of using macrophytes in ponds is that the pond effluent has a low TSS content compared to that of WSPs, which normally contains high quantity of algae (Al-Nozaily 2001; Awuah *et al.* 2004; Caicedo Bejarano 2005).

### 2.3.2. Macrophyte pond classification

The basic classification of macrophyte ponds is based on the mean of plant growth: emergent, submerged, free floating or rooted with floating leaves based ponds (Figure.2.2).



**Figure 2. 2 :** Schematic representation of different types of macrophyte pond (Brix, 1991, Vymazal, 2005)

Combined systems are systems in which different types of macrophyte are used, in the same pond or the association of different types of macrophyte ponds used in the same system.

Several aquatic plants have been tested for wastewater treatment; these plants, including floating (water hyacinth, water lettuce, duckweed), submerged (algae, *Lemna trisulca*), or emergent (*Cyperus papyrus*, bulrush, reed) have all shown their efficiency in removing pollutants from water (Brix 1997). The most used floating aquatic plants in wastewater treatment are water hyacinth, water lettuce and duckweed and several treatment plants using them exist in the world (Ho and Wong 1994; Perdomo *et al.* 1999; Al-Nozaily 2001; Caicedo Bejarano 2005; Awuah 2006; Zimmels *et al.* 2006; Alvarado *et al.* 2008; Zheng *et al.* 2013).

Water lettuce was reported to be the most common floating aquatic used for wastewater treatment in Africa (Koné 2002). The technology (with water lettuce) was introduced in Africa/ Cameroun in 1987 but it had the most application in Senegal and Burkina-Faso (Agendia *et al.* 1997; Koné 2002).

Water hyacinth is reported to be very productive which reflects their effectiveness in removing pollutant from wastewater, especially nutrients (Reddy and DeBusk 1987; Reddy and D'Angelo 1990; Polprasert *et al.* 1994; Polprasert and Khatiwada 1998; Koné *et al.* 2002; Awuah 2006). Another advantage of using water hyacinth is that these macrophytes can withstand high organic loading rates over 1000 mg O<sub>2</sub>/l of BOD<sub>5</sub> (Maharjan and Ming 2012). Nevertheless, the reported microbial and acidic quality of the effluent from WHPs call for the need of polishing before discharge or reuse (Polprasert *et al.* 1992; Kim and Kim 2000; Adewunmi *et al.* 2009; Maharjan and Ming 2012).

DWPs are reported to have an overall low cost due to the ease of their harvesting resulting from the simplicity of their morphology and physiology, their high nutritional value, their great biomass production and the huge number of reuse options that they offer (Skillicorn *et al.* 1993; Bonomo *et al.* 1997; van der Steen *et al.* 1998; Xu *et al.* 2011; Xu *et al.* 2012). Duckweed is probably one

of the best macrophytes to convert nutrient to biomass. The use of duckweed in ponds is a beneficial way to remove ammonia from wastewater, as this is their preferred form of nitrogen (Ingemarsson *et al.* 1987; Caicedo Bejarano 2005; Nasr *et al.* 2008). The DO content, the microbial and the pH of effluent of DWPs around 8 makes them suitable for discharge and reuse in agriculture (Al-Nozaily 2001; Caicedo Bejarano 2005; Awuah 2006). Yet, duckweed are reported sensitive to high organic loads with the related ammonia toxicity which can lead to a complete degeneration of the plants (Reed *et al.* 1995; Caicedo *et al.* 2000; Zimmo *et al.* 2005).

### **2.3.3. Water hyacinth and water hyacinth ponds**

Originating from Amazon Basin - South America, *Eichhornia crassipes* (Martius) Solms-Laubach, known as water hyacinth (waterhyacinth or water-hyacinth), is an erect, stoloniferous, free-floating perennial aquatic weed which has spread throughout the world (Gopal 1987; Center *et al.* 2002; Coetzee *et al.* 2007). Water hyacinth was first observed in West Africa in the late 1970s, as an ornamental plant because of its attractive pale blue or purple flower with yellow central patch. The plant was first reported in Benin in 1977 and about 10 years later became the major floating water weed in the south east of the country (Van Thielen *et al.* 1994; De Groote *et al.* 2003; Coetzee *et al.* 2009).

Water hyacinth (Figure 2.3), belongs to the small family of herbaceous monocotyledons *Pontederiaceae* which consists of six genera and 30 to 35 species (Center *et al.* 2002; Coetzee *et al.* 2009). The buoyant leaf differs in size and morphology depending on the growth conditions. Under low surface coverage conditions the kidney-shape leaf forms short (<30cm) and bulbous petioles allowing for a stable platform for vertical growth (Center *et al.* 2002; Coetzee *et al.* 2009), while in dense stands, elongated petioles (up to 1.5 m) are formed. The six to 10 leaves are arranged in whorls. Each leaf lasts up to 6 to 8 weeks before climax (aging) (Center and Spencer 1981; Center *et al.* 2002).



**Figure 2. 3 : Water hyacinth (Hounkpè 2013)**

Both the rhizome and the fibrous, feathery roots remain submerged. Sexual reproduction is limited by a scarcity of suitable pollinators and a lack of appropriate sites for germination and seedling establishment (Barrett 1980). The main mode of population increase is vegetative, via ramets (daughter plants) formed from axillary buds on stolons produced through elongation of internodes (Center and Spencer 1981). Once the ramets have developed roots, the stolons either decay or break, separating from the parent plant. Water hyacinth leaves can survive up to 6–8 weeks in water before senescence (Coetzee *et al.* 2009);

In the absence of its original suite of natural enemies, and usually in nutrient-enriched waters, *E. crassipes* populations increase rapidly, doubling under suitable conditions every 6 to 18 days (Edwards and Musil 1975; Williams and Hecky 2005; Coetzee *et al.* 2009). An increase in water area coverage by a factor of 1.012 to 1.077 per day has been estimated in some countries (Gopal 1987; Téllez *et al.* 2008) It has been reported that in favorable growth environments, a single plant can reproduce up to 140 million daughter plants each year, enough to cover an area of 1.40 km<sup>2</sup> with a fresh biomass of 28,000 tons (Ogutu-Ohwayo *et al.* 1997; Lu *et al.* 2007).

Water hyacinth is presented most of the time as the worst invasive aquatic weeds because they can become invasive in nutrient-enriched water and in absence of natural enemies. This is due to the long-lived seeds produced by their flowers, their fast spread and congested growth and their ability to cope easily with new environments forming expansive colonies of tall, interwoven plants (Wilson *et al.* 2000; Center *et al.* 2002; PD Gamage and ASAEDA 2004; Williams

and Hecky 2005; Malik 2007; Coetzee *et al.* 2009; Ndimele *et al.* 2011; Coetzee and Hill 2012). Water hyacinth seeds can remain viable for up to 20 years in sediments and can germinate on moist sediments or in warm shallow water (Matthews 1967; Gopal 1987; Center *et al.* 2002). The density of mature plants can reach 11,000 plants/m<sup>2</sup> (Nesic and Jovanovic 2010).

On the other hand, when looked from usefulness side, water hyacinth appears to be a valuable resource with several useful properties (Moreland *et al.* 1991; Sahu *et al.* 2002; Singhal and Rai 2003; Malik 2007; Nesic and Jovanovic 2010; Wang *et al.* 2012). This potential to double in biomass in a matter of days and their ability to reproduce successfully in new nutrient-enriched habitat make water hyacinth a good candidate for wastewater purification. Water hyacinth has been proved to be efficient in improving effluent quality from oxidation ponds and as a main component of an integrated advanced system for treatment of municipal, agricultural and industrial wastewaters (EPA 1988; Polprasert *et al.* 1992; Singhal and Rai 2003; Lu *et al.* 2008; Nesic and Jovanovic 2010; Chunkao *et al.* 2012).

In fact, the Iron Bridge water hyacinth system in Florida, USA was reported to remove 60% of BOD<sub>5</sub>, 43% of TSS and 35 to 80 % phosphorus (U.S. EPA,1988). For pig farm waste water as influent, removal rates of organic load were reported to be 74 -93% of COD in a small-scale WHP and 52% of COD in a pilot-scale at a loading rate at 200kg COD/ha/d and ten to twenty days retention time (Polprasert *et al.* 1992). This organic load removal reached 97% of BOD<sub>5</sub>, in addition of over 90% removal of phosphate and nitrogen, with a combined water hyacinth, duckweed and blue-green algae pond treating sewage (Sinha and Sinha 2000). Also, Lu, *et al* (2008) have reported, after monitoring a full scale plant of 688 m<sup>2</sup> treating wastewater from a duck farm for forty days, removal rates up to 64.44% of COD, 21.78% of TN and 23.02% of TP. The biomass produced was used efficiently to feed the ducks.

The absorption of nitrogen and phosphorus per hectare of water hyacinth has been estimated at 2,500 kg and 700 kg per year, respectively, if maximum growth could be sustained. The plants can also release, through their root

system, 2.4 to 9.6 g of oxygen per m<sup>2</sup> per day in the water (Kengne *et al.* 2000). Also, Reddy, *et al* (1982) observed, after one year field scale study, 78 to 81% of NO<sub>3</sub> - removal and 54% removal of soluble phosphate in 3.6 days from agricultural wastewater. These rates were 72% for nitrogen and 63% for phosphate when a WHP was used to treat dairy effluent (Tripathi and Upadhyay 2003). In a pilot system composed of a WSP followed by a WHP (to control algae in the effluent), receiving a secondary effluent in Korea, Yi, *et al* (2009) observed nitrification and denitrification rates of 0.04 and 0.02 g/kg day at 20 °C (wet weight basis), respectively. These rates were strongly affected by seasonal change (Yi *et al.* 2009).

However, water hyacinth required a ratio of nutrient and limit heavy metals content for an optimum growth. An experiment carried out in a batch reactor of 27 l in a greenhouse, using landfill leachate as influent, revealed that ratio N/P/K, total metal content and pH range for optimum growth of water hyacinth were respectively, 6.5/1.0/2.4, 0.10 mequiv /l. and 5.8 to 6.0 (El-Gendy *et al.* 2005).

Also, a WHP was tried as secondary treatment to remove algae from the effluent of WSP in a pilot scale plant. The WHP separated and successfully controlled the amount of algae cells. Also it was observed that the high pH from the WSP was adjusted in WHP and the effluent from the later was acidic (Kim and Kim 2000).

Moreover WHP, compared to WSP, has been reported more efficient in pollutant removal. A comparative study carried out showed that the presence of water hyacinth in a facultative pond improved its TKN removal rate which was 73.7% against 30% removal for a facultative pond without water hyacinth in the same period (Orth and Sapkota 1988). Sajn Slak *et al.* (2005) carried out a comparative study of a WSP and combined *Phragmites australis* and *Eichhornia crassipes* ponds, receiving a secondary effluent from a conventional process treating municipal and technological wastewater in Slovenia, at a loading rate of 0.13 m/d. They found the best reduction of TSS by 64.6%, TN by 38%, COD by 67.2% and BOD<sub>5</sub> by 72.1% with the macrophyte based pond with 4 day retention times.

Similar observations were made by Maharjan and Ming (2012) with an experimental study carried out in small scale WHP and WSP pilot ponds of 146 l at Nakkhu, Lalitpur, Nepal. In fact, the results from their experiment demonstrated that the best reductions in BOD<sub>5</sub> by 98.22%, TP by 72.79%, TN by 83.26% were observed in a four week period, in the WHP. Nevertheless the effluent obtained was not suitable for discharge or reuse, especially with regard to the microbial quality. However the plants were able to withstand BOD<sub>5</sub> over 1000 mg/l. (Maharjan and Ming 2012).

#### 2.3.4. Duckweed and duckweed ponds

Duckweed are the simplest smallest aquatic flowering plant (Hillman 1961). They belong to the scientific family of monocotyledon floating plants *Lemnaceae* which divide into 4 genera: *Lemna*, *Spirodela*, *Wolffia* and *Wolffiella* (Figure 2.4) and 28 species (Hillman 1961; Bonomo *et al.* 1997). This green plant can grow floating on the surface (*Lemna minor*, *Lemna gibba* and *Spirodela*), floating just below the surface (*Wolffiella*) or submerged (*Lemna trisulca*) (Al-Nozaily 2001).



**Figure 2. 4** : Duckweed (Wendeou *et al.* 2013)

Their leaves and stems are not distinguishable like in other vascular plants; they are fused to form the so called “fronds” (Caicedo Bejarano 2005). The frond size ranges from 2 to 6 mm for *Lemna*, up to 10 mm for *Spirodela* (Al-Nozaily 2001).

Duckweed has been grown on different qualities of wastewater (Harvey and Fox 1973; Al-Nozaily 2001; Caicedo Bejarano 2005; Awuah 2006; Xu *et al.* 2012). Duckweed reproduction is through the production of new fronds (“daughters”) from two meristemic regions (pockets) on each side of the narrower end of the

older fronds ("mothers") (Hillman 1961; Bonomo *et al.* 1997; Al-Nozaily 2001). The rate of reproduction can be influenced by the plant size but also by the biomass density and the medium composition (Bonomo *et al.* 1997; Driever *et al.* 2005).

Bonomo *et al.* (1997) derived their conclusions from literature review and experiment carried at Milan, Italy, in a pond of 450 m<sup>3</sup> treating a settled wastewater at a loading rate of 9 g/m<sup>3</sup>.day of BOD<sub>5</sub> with a retention time of sixteen days. The results revealed a relative growth rate of duckweed related to the initial mass of 0.1 to 0.35 g DW/ g .day corresponding to a mass doubling time of 2.3 to 7.3 days ; also they demonstrated that the plants can grow under temperature as low as 5 °C (Bonomo *et al.* 1997). Other studies have reported growth of 29g/m<sup>2</sup>.day for *Lemna minor* (Cheng *et al.* 2002) and 12.4 g DW/m<sup>2</sup>.day for *Spirodela polyrrhiza* (Xu *et al.* 2011). Al-Nozaily (2001) has obtained a maximum growth rate of 0.31 Wet Weight/day after studying, under laboratory condition at average temperature of 20°C, the growth kinetic of duckweed using artificial culture medium.

Due to its very high growth rates, one of the highest in the plant kingdom, duckweed can achieve high nutrient removal efficiencies. Efficiencies in TP removal were reported to range between 14 mg/m<sup>2</sup>.day and 74 mg/m<sup>2</sup>.day (63 to 99% of the initial TP) and that of TKN between 120 mg/m<sup>2</sup>.day and 590 mg/m<sup>2</sup>.day (73 to 97% of the TKN) (Körner and Vermaat 1998). However, this removal depends on the amount of the pollutant in solution and the retention time.

DWPs are efficient in reducing nutrient at highly variable removal rate. In fact, a study carried out in northern California with a pilot DWP of 10x30x1.0 m<sup>3</sup>, treating swine wastewater, and resulted in removal of 92.9 mmol/m<sup>2</sup>.day and 2.90 mmol/m<sup>2</sup>.day of N-NH<sub>4</sub> and P-PO<sub>4</sub><sup>3</sup> respectively (Xu *et al.* 2012). These removal rates from swine wastewater by *Lemna minor* obtained under ambient conditions were reported to reach 3.36 g/ m<sup>2</sup>.day for nitrogen and 0.20 g/ m<sup>2</sup>.day for phosphate when experiment was carried in vitro. The rates were 2.11

g/ m<sup>2</sup>.day for nitrogen and 0.59 g/ m<sup>2</sup>.day for phosphate in WHP under Californian ambient condition with a retention time of six weeks (Cheng *et al.* 2002). Similar experiments carried out under laboratory condition at constant temperature using *Spirodela punctate* in ponds of 300 l of artificial swine wastewater resulted in removal rates of 0.955 mg/l.h, 0.129 mg/l.h and 31.92 g/m<sup>2</sup>, respectively nitrogen, phosphorus and biomass production (Cheng *et al.* 2002). A duckweed pond in Benin (West Africa) has reached, in 10 days, a rate of removal from pre-treated sewage of 48.5 mg/m<sup>2</sup>.day for PO<sub>4</sub><sup>2-</sup>, 117 mg/m<sup>2</sup>.day for TKN, 83% for COD and 80.8% for turbidity (Wendeou *et al.* 2013). Removal rates of 48% and 50% were observed respectively for nitrogen and phosphorus with a series of seven pilot DWPs with three days retention time under ambient conditions with mean temperature 23.2°C and light intensity of 413 cal/ m<sup>2</sup>.day. (Caicedo Bejarano 2005).

Several scientists have demonstrated the efficiency of the potential of DWPs to remove organic loads and pathogens from wastewater (Alaerts *et al.* 1996; Al-Nozaily 2001; Zimmo *et al.* 2003; Caicedo Bejarano 2005; Awuah 2006; Nasr *et al.* 2008). In fact, the results of over four years study carried out in a full scale treatment plant with a DWP of 0.6 ha at Mirzapur, India (temperature between 19°C and 35°C), revealed removal rates between 90-97% for COD, 95-99% for BOD<sub>5</sub>, and 74-77% for TKN and TP. The DWPs were treating secondary effluent of domestic wastewater at a loading rate of 48-60 kg of BOD<sub>5</sub>/ha.day with a retention time of 20.4 days. The DWP effluent contained less than 2.7 mg/l of TKN and 0.4 mg/l of TP (Alaerts *et al.* 1996). Nasr, *et al.* (2008) confirmed these removal percentages with DWP of 1,920 cm<sup>2</sup> receiving anaerobically pretreated domestic in Egypt. In fact their removal rates ranged between 53.3 and 58.4% for COD, 58.6 and 66% for BOD<sub>5</sub>, 52.4 and 44.1% for TSS, 66.7 and 73.4% for nitrogen, 53.4 and 67.3% for phosphorus and 3 and 5 logs for FC at two retention times, ten and fifteen days (Nasr *et al.* 2008). FC removal rates in the above range were observed by Awuah (2006) and Awuah, *et al.* (2004) in their experiments carried under tropical condition in Ghana in a pilot small scale pilot plant with pretreated domestic wastewater, by Caicedo (2005) with an experiment carried

out under tropical conditions in Ginebra, Colombia, in full scale ponds, with 11.5 days retention time, receiving anaerobically treated effluent at a rate of 19.7 m<sup>3</sup>/day and by Zimmo, *et al* (2003) in a study carried out in pilot scale ponds in Jerusalem.

Comparative studies of WSPs and DWPs carried out by Awuah, *et al* (2004), Caicedo (2005) and Zimmo, *et al* (2003), in same conditions as above, revealed that DWPs were more efficient in removing TSS, nutrient and organic loads than WSP while the later had the best FC removal rate. However, they concluded that the effluent quality of the DWPs, with almost neutral pH, was suitable for reuse in agriculture and fish farming.

Nevertheless the complete decay of the duckweed observed by Zimmo, *et al* (2005) with wastewater containing over 100 mg/l of nitrogen as opposed to values for optimum growth ranging from 15 to 60 mg/l indicates that DWPs are only suitable for low strength wastewater, or for use as secondary or tertiary treatment (Bonomo *et al.* 1997; Zimmo *et al.* 2005).

## **2.4 Organic load, nutrient and pathogen removal mechanisms and recycling in macrophyte ponds**

In macrophyte ponds, the processes leading to wastewater purification include physical, chemical and biological processes such as chemical transformation of pollutants, oxidation, photo-oxidation and biodegradation of organic matter, settlement of suspended solid, chemical precipitation, breakdown, transformation and up take of pollutants and nutrients by microorganisms and plants, absorption and ion exchange on the surface of the plants, sediment and litter, predation and natural die off and settling of suspended particulate matter. These removal mechanism are not really different from the ones in algal ponds (EPA 1993; Bonomo *et al.* 1997; Renou 2006).

However, it has been proven, in particular through comparative studies of ponds with and without macrophytes, that the presence of macrophytes in

ponds improves their removal efficiency for suspended solids, organic loads and nutrients compared to waste stabilization ponds (WHO 1986; EPA 1993; Kivaisi 2001; Zimmo *et al.* 2002; Caicedo Bejarano 2005; Rodriguez and Jenssen 2005; Slak *et al.* 2005; Awuah 2006).

For instance, the roots of water hyacinths act like a living substrate for microorganism attachment and then provide a significant degree of treatment (EPA 1993). Also, the root system allows for microbial growth which makes the medium suitable for filtration and adsorption of suspended materials, nutrients and heavy metals (Center *et al.* 2002). Macrophytes meet their nutrient need for their multiplication by taking them up from the water through their roots, their stems or leafs (Brix 1997; Nasr *et al.* 2008).

Another attractive reason for using macrophytes such as duckweed and water hyacinths in wastewater treatment for resource recovery is the advantage of recycling the plant biomass as green fertilizer or animal food (Polprasert *et al.* 1994; Gunnarsson and Petersen 2007).

Due to their high vitamin A and nutrient content (up to 3.2% of dry matter and C/N ratio in the range of 15–35 ), water hyacinths can be used as animal food; these characteristics added to their high dry matter production make water them suitable for use as green manure in agriculture, and thus the potential loss of nutrients through drying or composting will be reduced (Abdelhamid and Gabr 1991; Gunnarsson and Petersen 2007). The use of water hyacinths as green manure for nutrient deficient soils has resulted in improved crop yields. A direct application of water hyacinths as green manure has been reported by Gunnarsson and Petersen (2007) based on a literature study, to be the best and cost effective alternative for small-scale use as proposed in the current research work. However, the rate of fertilizer production depends on the quantity of plants that can be harvested and then on the biomass production rate (Gunnarsson and Petersen 2007). Alternatively, water hyacinths are known to contain soft organic matter with a hemicellulose content in the range of 22% to 33.97% by dry weight, and a moisture content of 90 to 95% which are favourable

for energy production (biogas, ethanol, hydrogen or briquette) thereby combating deforestation (Singhal and Rai 2003; Jayaweera *et al.* 2007; Su *et al.* 2010; Okoli *et al.* 2011; Ganguly *et al.* 2012)

Duckweed has been reported to have one of the highest protein contents within the plant kingdom. Protein content under ideal conditions can reach up to 40%, with a high water content ranging between 94% and 95% of dry weight (Landolt 1986; Oron *et al.* 1986; Landesman *et al.* 2005). Compared with most other aquatic plants, duckweed has a greater potential because of their unique morphological and physiological properties (Xu *et al.* 2012). Duckweed can be used as agricultural fertilizer and in the production of high quality “green” compost because of its exceptionally high content of nitrogen, or in the conversion of biomass into organic fuel, if grown on domestic water lacking in heavy metals and other hazardous compounds (Bonomo *et al.* 1997; van der Steen *et al.* 1998; Caicedo Bejarano 2005; Xu *et al.* 2011; Xu *et al.* 2012). Due to its high content of vitamins, amino acids and enzymes and low content of fibres, duckweed is good as food for animals (Culley Jr and Epps 1973; Harvey and Fox 1973; Mbagwu and Adeniji 1988; Caicedo Bejarano 2005).

## **2.5 Metal removal mechanism in macrophyte ponds**

### **2.5.1. Metal toxicity and sources in wastewater**

Metals, especially heavy metals, are a potential risk to public health. They may be highly toxic, carcinogenic, teratogenic, mutagenic and may concentrate in and damage the liver, kidneys, pancreas and thyroid; some heavy metals can increase blood pressure and provoke nerve block (Singhakant *et al.* 2009; Dotro *et al.* 2010; Yadav *et al.* 2010; Lizama A *et al.* 2011). Due to the fact that metals do not decay like organic matter, they are potentially persistent in the environment and readily accumulated to toxic levels (Chung *et al.* 2011).

Arsenic, cobalt, manganese, chromium, selenium, antimony, chromium, zinc and iron accumulation in crops and vegetables has been observed at high

concentration in some areas of Thailand where untreated contaminated wastewater was used for irrigation. Chung, et al (Chung *et al.* 2011) has shown that irrigation with domestic wastewater increases the concentration of metals (lead, cadmium, copper, and zinc) in irrigated soil. It has also been proved that cereal grains such as maize, wheat and brown rice can take up metals through contaminated soil (Cubadda *et al.* 2005; Chung *et al.* 2011; Guo *et al.* 2011; Si *et al.* 2011) leading to direct food contamination. Metal accumulation in plants has been demonstrated by several scientists (Gnamuš *et al.* 2000; Manios *et al.* 2002; Comino *et al.* 2009); this accumulation may result in food chain contamination due to the fact that metals can be transferred from plants to herbivores and then to carnivores (Gnamuš *et al.* 2000).

Metal toxicity in plants results in the inhibition of several enzymes by blocking their activity or changing their structure (Bidar *et al.* 2007). This toxicity also affects their photosynthetic system (Vassilev *et al.*, 1995. Singh *et al.*, 1997. Dong *et al.*, 2005.). Heavy metals or metals with atomic weights greater than sodium and atomic numbers greater than 20 also exert adverse effects on the enzymatic activities of microorganisms (Huynh, 2009). They cause protein denaturation or destruction of the integrity of the cell membrane, thereby affecting the growth, the morphology and the metabolism of microorganisms (Leita *et al.* 1995). This reduces their performance in pollutant removal in biological wastewater treatment processes.

Heavy metals can be found at variable and significant concentrations in natural waters as a result of natural phenomena in the geo-cycle of matter (erosion and weathering of soils and rocks, volcanism, air transport) (Burnol *et al.*, 2006. Bidar, 2007; Chikhi, 2008 Huynh, 2009).

Most metals found in wastewater are from human agricultural and domestic activities but most of them are produced by industrial activities (Bidar *et al.* 2007).

Metal pollution of domestic wastewater comes from the combustion of fossil fuels (oil, coal) and wood, household wastes (storage and combustion), vehicle emissions (Bidar *et al.* 2007) as well as faeces, pharmaceutical and cosmetic products, cleaning chemicals and paint (Blais, 1992; Benyahia-Kafi, 2006). Various metals, such as iron, aluminium, copper, zinc, magnesium, manganese, lead, cadmium and mercury are found in domestic wastewater. The concentration of metals in domestic wastewater is generally assumed low (lower than the harmful level) compared with that of industrial wastewater (Kafi Benyahia 2006). For this reason, it is not usually the first concern in treating domestic wastewater, but care must be taken if the effluent is to be reused in agriculture or aquaculture in order to prevent food chain contamination (Sridhar and Sharma 1985; Lesage *et al.* 2007; Liu *et al.* 2007; Comino *et al.* 2009). However in developing countries, due to domestic micro-industries and unsafe disposal of certain cosmetic products, batteries and cleaning chemicals, metals such as zinc, iron and copper can occur in sewage at concentrations near that of the industrial wastewater (Hounkpè *et al.* 2013).

Although copper, iron and zinc are micronutrients for plants and other living-organisms, they are highly toxic when the concentration exceeds a certain level (Huynh 2009). The toxicity of copper is quite important for living organisms at relatively low concentration while that of zinc and iron occurs at moderate concentrations (Bidar *et al.* 2007) (Noppe (1996) et al Burnol beings. (2006) Bidar (2007)).

Heavy metals such as iron, copper and zinc were reported commonly present in quite huge quantity, over 20 mg/l for each metal, in domestic sewage or septage of Sub-Saharan cities (Hounkpè *et al.* 2013; Hounkpè *et al.* 2014).

### **2.5.2. Metal removal in water hyacinth ponds**

Various chemical, physical and biological technologies exist as remediation methods for waste water contaminated with heavy metals. These include chemical precipitation, oxidation, coagulation-flocculation-sedimentation,

membrane filtration, ion exchange and adsorption. These technologies remain limited in use for the removal of heavy metals from domestic wastewater due to the relatively high cost of chemicals and materials and the periodic material regeneration cost in case of adsorption (Ibn Ghazala 2009; Joseph 2009).

Considering the ability of water hyacinth to grow under completely nutrient-poor conditions which makes it a promising candidate for metal removal from aqueous solution (Gamage and Asaeda 2005; Jayaweera *et al.* 2007; Malik 2007), several scientific studies have examined the performance and mechanism for the removal of metals by water hyacinths (Delgado *et al.* 1993; Liu *et al.* 2007; Jayaweera *et al.* 2008; Mishra and Tripathi 2008; Smolyakov 2012).

In macrophyte ponds, metal can be removed by precipitation, sedimentation and accumulation (Vymazal and Krasa 2003; Visermanee *et al.* 2008; Comino *et al.* 2009; Lizama A *et al.* 2011; Si *et al.* 2011), although a considerable amount of metals in the wastewater is taken up and accumulated in the macrophytes during treatment (Liu *et al.* 2007; Comino *et al.* 2009; Lizama A *et al.* 2011). The high plasticity of water hyacinth root morphology (Xie and Yu 2003) conferred also to them a high capacity for sequestering heavy metals, rare earth elements, and other chemicals and providing sites for precipitation (Hadad *et al.* 2006; Lesage *et al.* 2007; Lu *et al.* 2007).

Water hyacinths have demonstrated their efficiency and their ability to tolerate a wide range of selected metals (iron, zinc, copper, aluminium, chromium, zinc, cadmium, lead, selenium, caesium and cobalt) by taking them up from contaminated water through roots and leaves; therefore they can probably be used for the removal of heavy metals, even at large scale. (Soltan and Rashed 2003; Sheoran and Sheoran 2006; Hasan *et al.* 2007; Jayaweera *et al.* 2007; Jayaweera *et al.* 2008; Gakwavu *et al.* 2012; Saleh 2012; Smolyakov 2012).

Indeed, Delgado, *et al.* (1993) reported a complete removal of zinc, chromium and cadmium after 24 days of plant growth in a metal-containing aquarium of 8 l in greenhouse at temperatures lying between 28-30°. They observed phyto-

toxicity symptoms at cadmium concentration above 2.5 ppm and at 9 ppm of chromium and zinc. A removal rate by water hyacinth of 600 mg As/ha.day (Alvarado *et al.* 2008) and the potential use of the plant to remove As from drinking water at the household level (Misbahuddin and Fariduddin 2002) have also been reported.

Mishra and Tripathi (2008) reported a removal rate over 80% of  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cr}^{3+}$  and  $\text{Cd}^{2+}$  by water hyacinths after 15 days of retention. From that study, it came out that the iron was the most accumulated metal in the plant roots and the accumulation rate increased with the concentration of metal in the culture medium. Also, these researchers found that chromium at 10.0 and 20.0 mg/l was toxic while no adverse effect was observed for removal of zinc. The removal rate of copper decreased with the increase of the metal concentration in solution. (Mishra and Tripathi 2008).

Water hyacinth has selectivity and a great difference in accumulating various heavy metals; the removal rate depends on pH (Soltan and Rashed 2003; Smolyakov 2012). Smolyakov (2012) has reported removal rates of 92% and 76% for copper, 89% and 74% for lead, 76% and 50% for cadmium, and 82% and 43% for zinc at pH 8 and pH 6, respectively, from water containing an initial concentration of 500  $\mu\text{g/l}$  of zinc, 250  $\mu\text{g/l}$  of copper, 250  $\mu\text{g/l}$  lead and 50  $\mu\text{g/l}$ . They observed that the reduction was in the order lead approximate to  $\text{Cu} \gg \text{Cd}$  approximate to Zn in the first days of treatment (Smolyakov 2012).

Water hyacinth's ability to up-take metal from aqueous solution may depend on the nutrient concentration of the medium. In fact, an experiment carried out with synthetic Fe-rich industrial wastewater, under batch conditions, for a period of 15 weeks in an outdoor tropical environment, showed the highest phytoremediation efficiency of iron of 47% and the best accumulation of 6707 Fe mg/kg dry weight at the sixth week. This best accumulation was observed under completely nutrient-poor conditions (Jayaweera *et al.* 2008).

Water hyacinth is able to take up several metals together (Hasan *et al.* 2007; Smolyakov 2012). In fact, Hasan, *et al.* (2007) have tested, in experiments conducted in a container with individual plants in 1 l tap water containing Hoagland's solution, the removal of cadmium (1.0, 2.0, 2.5, 4.0 and 6.0 ppm), zinc (2.0, 4.0, 6.0, 8.0 and 12.0 ppm) and mixture of metal cadmium at 6.0 ppm and zinc at 12.0 ppm. They concluded that zinc and cadmium were successfully removed from aqueous solutions containing the metal alone as well as their admixture, even though metal uptake rate in the mixture was slower than that in the case of individual metals.

All these studies showed the ability of water hyacinth to accumulate heavy metals; however, due to the low concentration of metals in the most of the culture media used the real toxicity of metals such as iron, copper and zinc, are poorly developed. Also, the effect of these accumulations on the subsequent treatment and the possibility of contamination of the effluent by water hyacinth which has accumulated high quantity of metals through release in the medium have not been checked.

Considering the high concentrations (over 20mg/L) of these metals in domestic wastewater from some urban areas of developing countries (Cotonou for instance) (Hounkpè *et al.* 2013) and the risk of treatment plant effluent may be affected, further investigation should be carried when the plant have to be used for treatment of wastewater and nutrient recovery.

## **2.6 Factors affecting plant growth and performance in macrophytes ponds**

The treatment performance of macrophytes is highly related to their growth and multiplication. Unfortunately, as for all biological process, the growth of macrophytes and then their wastewater purification potential are influenced by a number of factors, such as nutrient availability, pH, light intensity,

temperature, organic loading rate, the dissolved oxygen content and the salinity (Gopal 1987; Center *et al.* 2002; Williams *et al.* 2005; Coetzee *et al.* 2009).

### **2.6.1. Temperature**

Warm temperature is beneficial for wastewater treatment, especially biological processes, as it allows the development of the microorganisms responsible for pollutant removal. The temperature enhances microbial activity and is involved in nitrogen reduction process. Below 10 °C and above 30 °C, the nitrifying activity drops rapidly (Ndiouck, 2006).

The optimum temperature for duckweed growth ranges between 17°C and 29°C (White 1936; Wedge and Burris 1982; Al-Nozaily 2001; Wilson *et al.* 2005). Duckweed can double its total frond number in four days when grown in secondary wastewater effluent at constant temperature of 24°C and light intensity of 190  $\mu\text{mol}/\text{m}^2\cdot\text{s}$  with 12 hour dark and light photoperiods (Harvey and Fox 1973).

Neutral pH, warm temperature and high light intensities favour water hyacinth proliferation (Gopal 1987; Center *et al.* 2002; Allgayer 2006; Téllez *et al.* 2008). Optimal water hyacinth growth occurs at temperatures of 22–30°C, while growth ceases when water temperatures drop below 10°C or rise above 40°C (Gopal 1987; Allgayer 2006). Under stress conditions, the plant uses the carbohydrates stored in the stem as source of energy, but prolonged cold temperatures, below 5°C, result in death of the plants (Gopal 1987; Owens and Madsen 1995; Sooknah and Wilkie 2004). The higher the temperature, the higher is the need for plants to close their stomata openings to the outside in order to avoid excessive water loss. This stomatal closure results in reduced gas exchange, and then the depletion of carbon dioxide and accumulation of the oxygen produced by photosynthesis (Gopal 1987).

### **2.6.2. pH**

Water pH affects many biochemical processes involved in macrophyte growth and metabolism, including the bioavailabilities of carbon dioxide for photosynthesis and the availability and absorption of nutrient ions (Park et al. , 2011, García et al, 2000. Craggs, 2005; Heubeck et al, 2007. Park and Craggs, 2010). The pH can affect the availability of essential minerals (phosphate, iron, molybdenum, zinc, manganese) or the solubility of toxic substances (McLay 1976). Low pH increases the risk of the presence of metals (copper, for example) in a more toxic ion form. High pH increases the concentrations of toxic ammonia (Pichard *et al.* 2005; Bisson *et al.* 2009).

The lower limits, optimum and upper limits pH have been respectively estimated for each duckweed species to be: *Wolffia*, pH 4-5-10, *Lemna* pH 4-6.2-10, *Spirodela* pH 3-7-10. *Lemna* was able to survive at a pH 3 even though the frond size was smaller (McLay 1976).

Water hyacinth can tolerate pH ranging between 4 and 10. A pH out of this range can inhibit the plant growth (Gopal 1987; Center *et al.* 2002; Allgayer 2006; Téllez *et al.* 2008). The pH can significantly inhibit water hyacinth growth due to deficiencies in nitrogen stripping at high pH (Azov and Goldman 1982).

However, the direct influence of pH on the wastewater purification performance of water hyacinth has not been investigated.

### **2.6.3. Light intensity**

The performance of macrophyte ponds is highly related to light intensity as it governs the photosynthesis activity of the plants. When the light intensity is out of the optimum range, a phenomenon of photoinhibition occurs. Damage is then caused to photosynthetic systems (light receptors), resulting in a reduction in pigment yield and then the slowing of the plant photosynthetic activity and productivity (Tillett 1988; Walker 2002; Walker 2009; Baya 2012).

The length of the light and dark periods at which the culture is exposed every day (photoperiod) plays an important role in the life processes of the plant. A variation in the photoperiod may significantly affect many physiological and ecological activities such as growth, reproduction, migration, and flowering. A short photoperiod can delay macrophyte development, while a photoperiod of 18 hours may inhibit the growth (Baya 2012).

The saturation light intensity value for most duckweed was reported to be approximately 300  $\mu\text{mol}/\text{m}^2$  (White 1936; Wedge and Burris 1982; Al-Nozaily 2001; Wilson *et al.* 2005). Growth rate up to 0.176/day and BOD<sub>5</sub> removal from water of 1084.63 mg/m<sup>2</sup>.day have been observed under a natural tropical climate with an average light intensity of 110 $\mu\text{mol}/\text{m}^2$  (Wendeou *et al.* 2013). Increases in relative growth rate (related to number of fronds) from 0.20/day and 0.19/day at light intensity of 60-70  $\mu\text{mol}/\text{m}^2$  to 0.28/day and 0.27/day, respectively for *L. minor* and *L. gibba*, at light intensity of 260-270  $\mu\text{mol}/\text{m}^2$  have been reported (Al-Nozaily 2001).

The fastest growth of water hyacinth was observed at 240 000 lux, but the plant minimum requirement has been stated to be at 24 000 lux (Téllez *et al.* 2008). However, the change in purification behaviour of water hyacinth at different light intensity remains unknown.

#### **2.6.4. Conductivity and salinity**

Duckweed is resistant to high conductivity (Caicedo Bejarano 2005). Xu, *et al.* (Xu *et al.* 2012) observed growth of healthy duckweed at a conductivity of 1460  $\mu\text{S}/\text{cm}$ . The optimum electric conductivity for duckweed growth has been reported to be between 600 and 1400  $\mu\text{S}/\text{cm}$  which is salinity between 430mg/l and 1062 mg/l (Wendeou *et al.* 2013).

Conductivity has been reported to be the main obstacle for the growth of water hyacinth (Téllez *et al.* 2008).

### **2.6.5. Dissolved oxygen and plant coverage density**

The coverage level of macrophytes in ponds affects the dissolved oxygen and plant growth. Dense mats of vegetation reduce the oxygen penetration in the medium; thus an increase in plant coverage leads to dissolved oxygen depletion in a pond, which inhibits plant growth (Bonomo *et al.* 1997; Center *et al.* 2002; Sengupta *et al.* 2010). In fact, negative growth of *Lemna minor* was reported at biomass coverage densities over 180 g of dry weight / m<sup>2</sup> when grown in a nutrient enriched medium at 23 ° C under laboratory conditions (Driever *et al.* 2005).

However, it has been reported that the oxygen consumption of macrophytes decreased with culture age (Hillman 1961; van der Steen *et al.* 1998).

### **2.6.6. Organic loads and nutrients**

Macrophyte growth is directly correlated with nutrient concentrations (Miyazaki *et al.* 1984; Gopal 1987). As nitrogen and phosphorus increase in concentration, plant biomass accumulation increases too (Gossett and Norris 1971; Reddy *et al.* 1989; Reddy *et al.* 1990). Zimmo, *et al.* (2005) reported a complete decay of duckweed in ponds when the nitrogen content of wastewater was over 100mg/L (Zimmo *et al.* 2005).

Several studies have reported that a high organic loading rate on macrophyte ponds can lead to ammonia toxicity, especially for duckweed, resulting in plant degeneration (Wang 1990; Clément and Merlin 1995; Caicedo *et al.* 2000; Zimmo *et al.* 2005).

As for the effect of the fluctuating organic loading rate on macrophyte pond system treatment efficiency, no literature has been found.

## **2.7 Macrophytes ponds and mosquito breeding**

The choice of a macrophytes process remains a taboo subject when making decision on wastewater treatment due to the risk of mosquitoes and other

parasites breeding and the suspected harm to human health. However, the use of macrophyte ponds does not necessarily mean the resurgence of malaria.

In fact, it was concluded, from a study carried out over a year in a macrophyte based treatment plant of about 1000 m<sup>2</sup> treating 45 m<sup>3</sup>/day of domestic wastewater from a residential area of Yaoundé (Cameroun), that the malaria vector mosquito (*Anopheles*) does not breed in macrophyte ponds. The major types of mosquito on macrophyte ponds were *Mansonia* and *Culex* which are not a malaria vector mosquito (Kengne *et al.* 2003).

This finding was confirmed by studies carried out in Ghana on a series of four ponds of water lettuce, duckweed and algae laid in parallel, during which *Culex* species were the only mosquitoes found. This study showed that duckweed contributes to the elimination of mosquito breeding in ponds, as during the experiment, apart from the second pond, the duckweed ponds had no mosquitoes larva. The mosquito larvae populations of 96/m<sup>2</sup>, 3,516/m<sup>2</sup> and 11,175/m<sup>2</sup> in duckweed, algal and water lettuce ponds, respectively were reported (Awuah 2006).

## 2.8 Concluding Discussion

The analysis of literature WHP and DWP shows that they are efficient in removing TSS, organic loads and nutrient from water. Also, DWPs have been shown to be efficient in removing pathogens from wastewater. Nevertheless, studies demonstrated the limits on DWPs to withstand high organic loading rates and the need of the effluent to be polished before discharge or reuse, due its acidic and microbial quality. Further observations indicated that WHPs are suitable for primary and secondary treatment while DWPs can be used as secondary or tertiary treatment of wastewater, especially high strength wastewater.

On the other hand, most of the reported studies on WHPs and DWP, showing their efficiency, were carried under batch conditions, at constant loading rate or ponds with few variations of the loading rate per experimental period.

No attempt has been made to understand the effect of fluctuating loading as can occur under conditions found in developing countries, where wastewater of different qualities, with high metal contents, may be discharged intermittently from trucks.

However, studies have demonstrated the ability of water hyacinths to take up heavy metals from water and accumulate them in their tissues. However the effect of such accumulation on the subsequent treatment performance (organic load and nutrient removal for instance) and the effluent re-pollution risk related to metal release have not been studied.

Studies have shown that macrophyte pond performance is related to plant growth. However, several factors such as temperature, pH, light intensity, conductivity, organic load and nutrient content of the medium affect this. Water hyacinth has been reported as very sensitive to pH and light intensity, but the effect of these parameters on overall pond performance has not been tested in the literature.

In order to take advantage of WHP and DWP qualities and at the same time to overcome their limits, this current research is proposing the use of a combined WHP and DWP system to treat and recover resources from wastewater.

In the process of development of such a combined technology, further research will be conducted to study the effect of fluctuating organic loading rate on such a system, and to gain an understanding on the effect of changing parameters such as pH, light intensity and metal content on pond performance and biomass production, especially regarding the water hyacinth, which more exposed to these changes.

# 3

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## ***Materials and methods***

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### **3.1 Introduction**

In order to carry out the present work focused in understanding the removal mechanisms of pollutants by macrophyte ponds and their ability for resources recovery, different set-ups of pond systems, influent solutions, analytical instruments and methods were used.

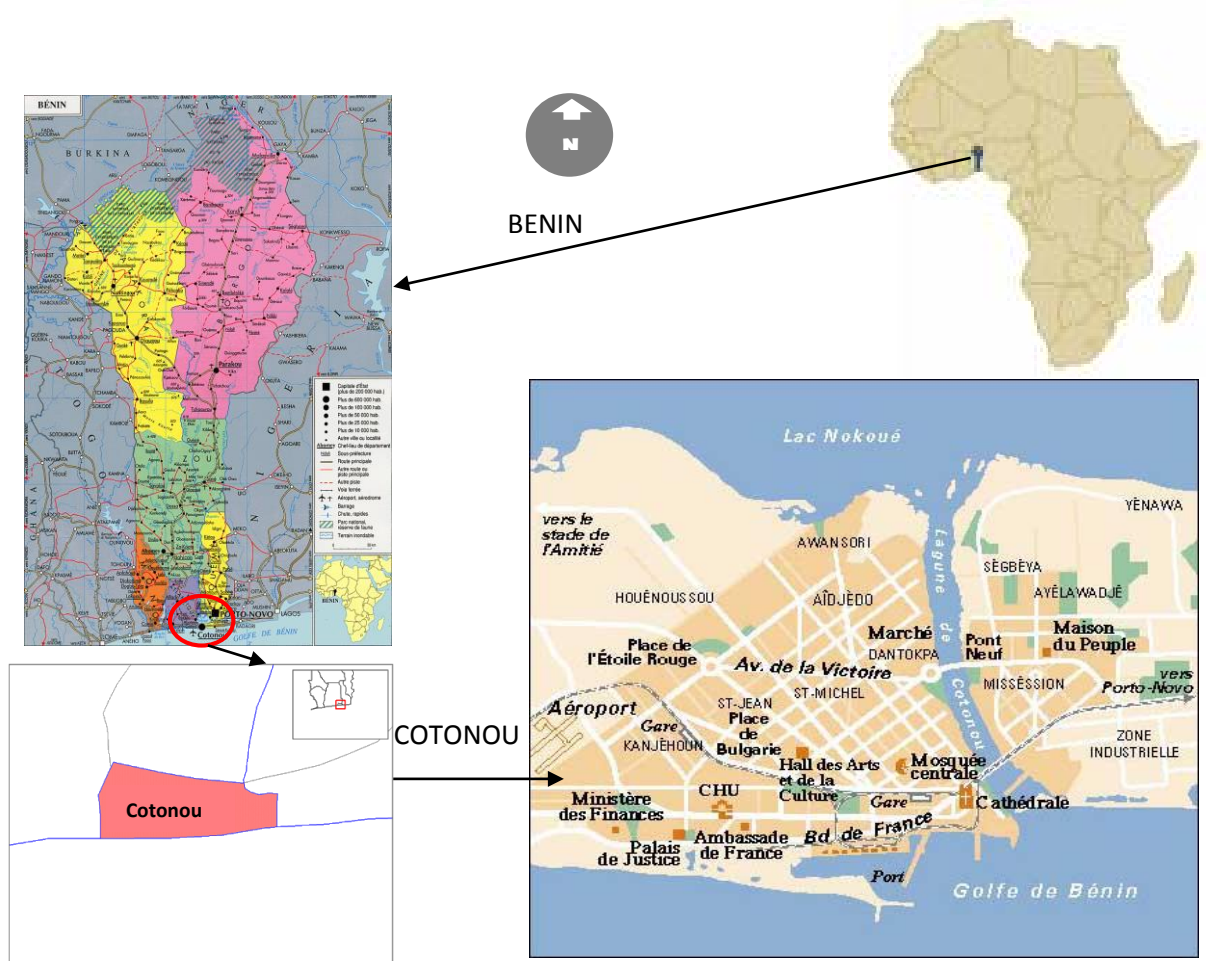
This chapter has two main objectives. The first one is to describe the macrophyte pond systems proposed to study pollutants removal and resource recovery. The second objective is to describe and characterise the materials (ponds, plants, media, chemicals, etc.), the instruments used and the protocols adopted to study the removal process and mechanisms occurring in ponds.

This chapter, named materials and methods presents brief description of the study site, systems design, set-ups and analysis methods used in the study. Section 3.2 describes the study site while the section 3.3 describes the different experimental set-ups. The section 3.4 presents the operational conditions and other auxiliary experiment such as fish culturing was also documented in this section. Section 3.5 documents risk assessment prepared and safety measures taken for the research. Section 3.6 describes various analytical methods used for the water quality variables determinations and section 3.7 presents the results analysis methods.

### 3.2 Study site description

- *General description of the city*

The study is conducted in Benin, one of the smallest countries in West Africa, located near the equator and between the parallels 6°30' and 12°30' of latitude and 1° and 3°40' of longitude. Benin, with an area of about 112 622 km<sup>2</sup>, and an estimated population of 9 983 884 inhabitants and growth rate of 3.25% according to the general census of the population and houses (RGPH) of May 2013, is bounded on the north by the river Niger, on the north west by the Burkina-Faso, on the west by Togo, on the south by the Atlantic Ocean and on the east by Nigeria (figure 2.1) (Bénin ; Bénin 2013).



**Figure 3. 1 : Map of Study area (adapted from(Bénin), 2013)**

The southern part of Benin is in the Sub-equatorial zone where rainfall is bimodal (April to mid-July and mid-September to October) with two dry

seasons (mid-July to mid-September; November to mid-March). During the rainy seasons, the annual rainfall recorded at the station at Cotonou Airport from 1953 and 2007 varied from 719.4 mm to 2470.2 mm with an average of 1308mm for that period of time. The minimum temperatures ranged from 23 to 26°C and the maximum temperatures from 28 to 32°C, with an annual average sunshine period of 2290 hours. The relative humidity is between 60% and 90% with an annual average relative humidity of 75% for Cotonou. The city is on a plain with a sandy soil having a porosity ranging from 35% to 40% and an infiltration capacity from 7% to 20%, depending on the level of the shallow groundwater table (ASCECNA 2008; Hounkpè *et al.* 2014).

Cotonou, with a population density of about 8600 inhabitants/km<sup>2</sup> and a surface area of 79 km<sup>2</sup>, is the biggest city of Benin where most of the political, administrative, economic, industrial, cultural and tourist activities are concentrated. Due to its attractive socio-economic situation and its limited area, the city is expanding to the surrounding municipalities Abomey-Calavi, Ouidah and Seme-Kpodji. This set of cities, known as “Le Grand Cotonou” (Greater Cotonou), covers 1% of the territory and counts more than 17% of the country’s total population (INSAE-BENIN 2002; Bénin 2013).

The common type of dwelling for households in the city is rooms in a compound (79%), with shared sanitation facilities (INSAE-BENIN 2002). The sanitation facilities most commonly encountered are primary types such as pit latrines or septic tanks (83% of the population) with shallow depth and high risk of groundwater pollution as they are not impervious. This groundwater is used, through wells, by 81% of houses for domestic purposes and most importantly by 5% of houses as drinking water with subsequent health problems. It has been reported that more than 85% of the grey water generated in the city is disposed of in the environment without undergoing any treatment (Hounkpè *et al.* 2014). The wastewater generated and the septages from those primary sanitation facilities are quite strong (Table 3.1). This Table 3.1 presents also the quality of

sewage and sullage of the university campus of Abomey-Calavi described below.

**Table 3. 1 : Greywater, wastewater and septage characteristics of Cotonou and Abomey-Calavi University adapted from (Hounkpè et al. 2013)**

Parameters	Unit	Sewage (UAC)	Sullage (UAC)	Septage (Cotonou)
Dissolved O <sub>2</sub>	mg/l	0.5 ± 0.13	0.8 ± 0.21	0.275 ± .096
Temperature	°C	29± 1.4	29.9 ± 0.5	29.1 ± 0.3
pH	U pH	6.8± 0.4	6.8 ± 1.0	7.5 ± 0.5
Conductivity	µS/cm	1 357± 530	1 092 ± 469	27 275 ± 10 802
Salinity	mg/l	1 247± 576	987 ± 669	23 196 ± 9 186
TDS	mg/l	812 ± 330	431 ± 149	13 640 ± 5 326
e <sub>H</sub>	mV	149 110	229 ± 60	-19 ± 27
Turbidity	NTU	133 ± 105		260 ± 103
Suspended Solids (SS)	mg/l	274 ± 152	90 ± 14	14 327 ± 12 372
COD	mg /l	790 ± 444	77 ± 6	28 227 ± 19 375
BOD <sub>5</sub>	mg /l	337 ± 148	60 ± 11	5 313 ± 3 184
NH <sub>4</sub> <sup>+</sup>	mg/l	16 ± 6	0.1 ± 0.1	155.3 ± 131
NO <sub>2</sub> <sup>-</sup>	mg/l	0.10 ± 0.04	0,04 ± 0.03	- -
NO <sub>3</sub> <sup>-</sup>	mg/l	84 ± 40	45 ± 14	5 075 ± 1 307
NTK	mg de N/l	103 ± 19	23 ± 5	
PO <sub>4</sub> <sup>3-</sup>	mg/l	14 ± 5	26 ± 24	759 ± 439
P total	mg/l	27 ± 3	13 ± 12	ND
Iron	mg/l	0.73	ND	41.87 ± 45.34
Zinc	mg/l	0.09	ND	11.19 ± 11.20
Copper	mg/l	0.03	ND	22.44 ± 22.61
Aluminium	mg/l	0.80	ND	34.33 ± 17.79
Manganese	mg/l	0.02	ND	4.00 ± 4.27
Lead	mg/l	ND	ND	0.25 ± 0.03
Cadmium	mg/l	ND	ND	0.02 ± 0.01
Faecal Coliforms	10 <sup>5</sup> /100ml	71.68± 20.95	3,15 ± 2.97	1 238 ± 1 219
Escherichia coli	10 <sup>5</sup> /100ml	61 ± 19	2 ± 3	951 ± 932
Faecal Streptococcus	10 <sup>5</sup> /100ml	3.3 ± 0.8	1,7 ± 0.18	ND
Total Coliforms	10 <sup>7</sup> /100ml	50 ± 15	1,5 ± 1.94	ND

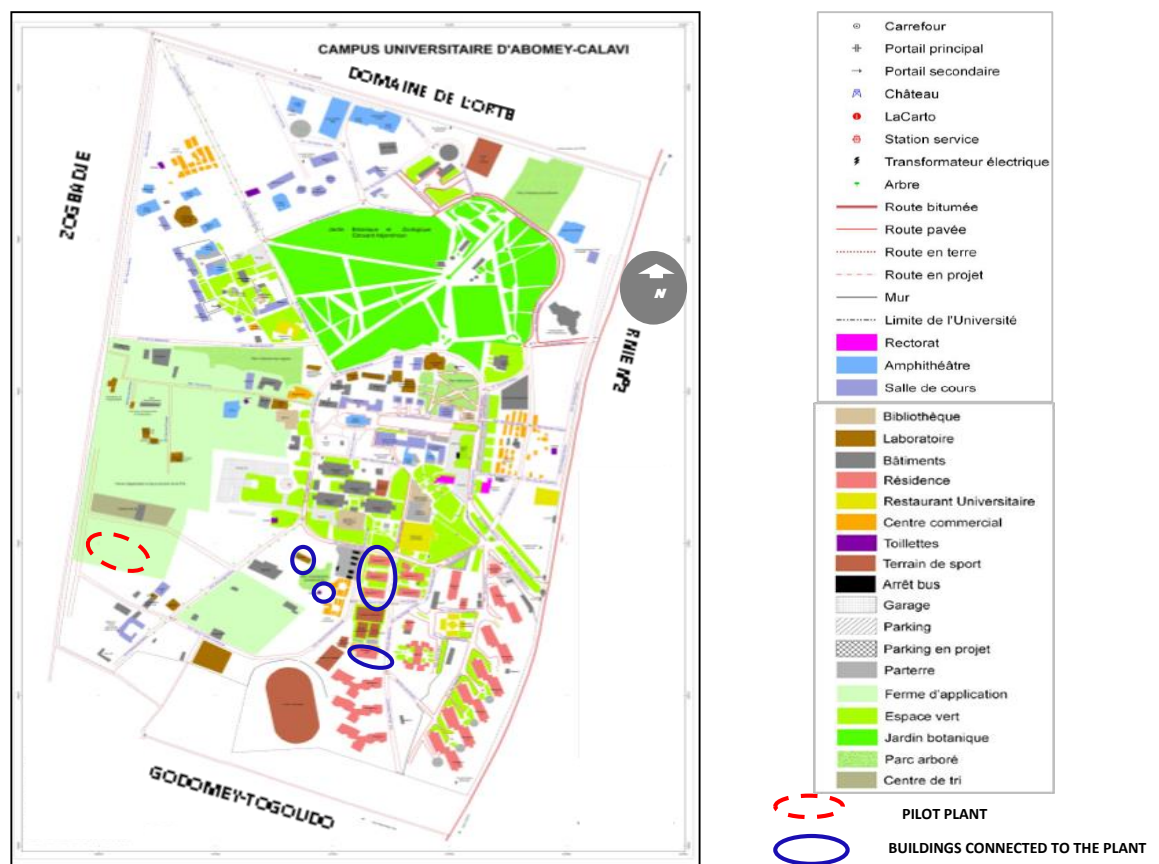
Value = mean ± standard deviation

ND = Non Determined

- *Description of the site for the experimental study*

The pilot scale system, used in the present study, is constructed on the University campus of Abomey-Calavi located at the entrance of the City Abomey-Calavi.

Created by the decree N° 70-217/CP/MEN of 21<sup>st</sup> August 1970 to receive 6000 students, the former National University of Benin (UNB), the current UAC, counted in 54 090 registered students in 2009, with an annual growth of student population of 17%. 4 558 students were accommodated in eleven university halls located on the Campus in 2010 (COUS-AC 2010; UAC 2010).



**Figure 3. 2 :** Pilot plant location on the University campus (adapted from (Bénin ; Cruz et al. 2013))

Five of the existing eleven existing university halls and one administrative building were connected by a low diameter sewer to the pilot plant. Figure 3.2

shows the Campus map and the location of the pilot plant and the buildings connected to it.

The pilot plant was used to treat wastewater generated from these halls. The generated wastewater qualities are presented in Table 3.1.

The climate conditions on the campus of UAC were the same as those observed in the city of Cotonou.

### **3.3 Experimental set up**

Two experimental set-ups were used for the present research work: systems of mini-ponds and a pilot plant.

#### **3.3.1. Mini ponds**

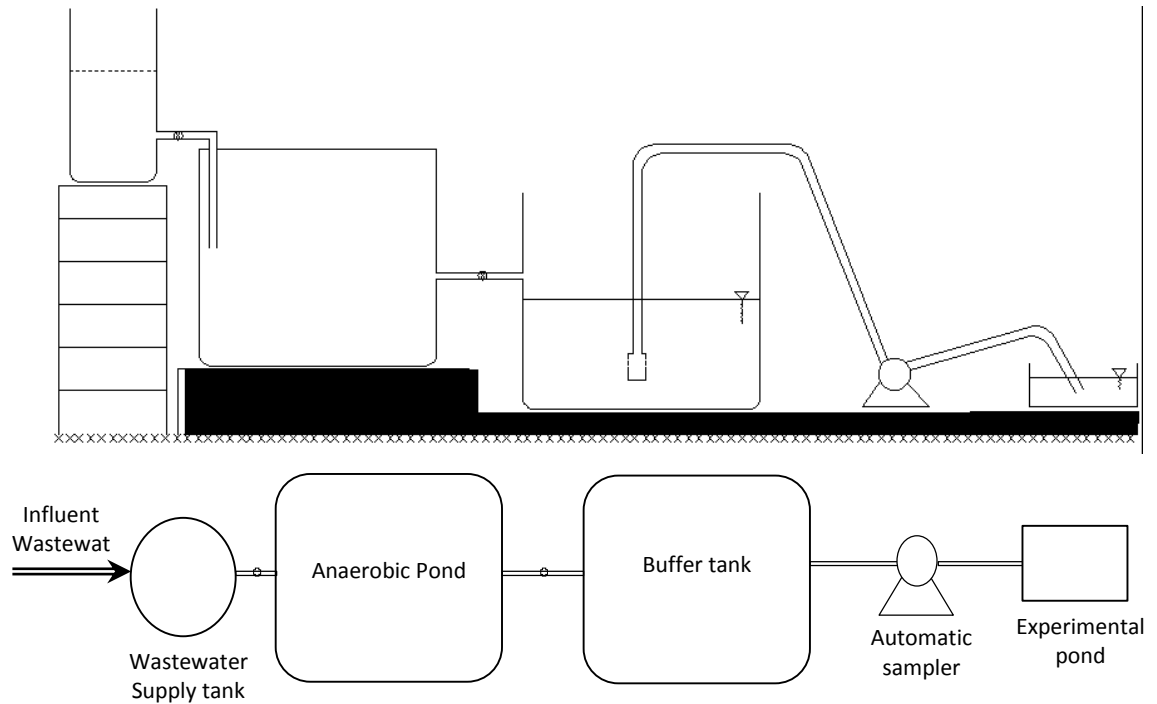
Three types of mini-ponds were used for the experiments, two types of mini-ponds using wastewater as influent water and a third type, in which an artificial culture was used.

- *Set-up with mini-ponds with wastewater as influent medium*

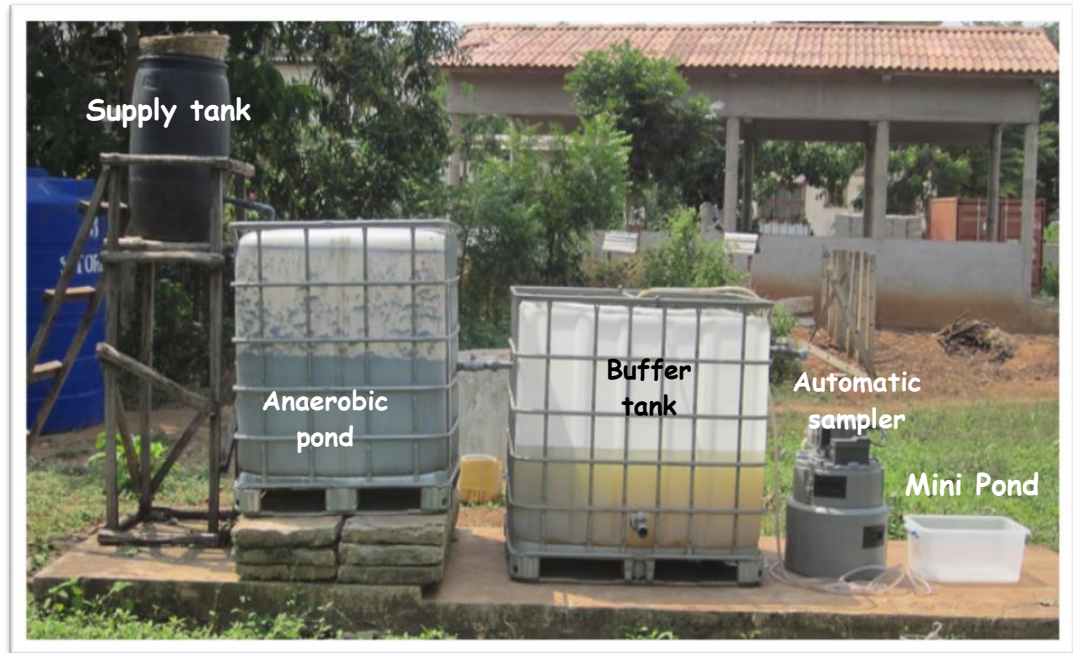
Using wastewater as medium, the experimental set up was composed, of a wastewater supply tank, a buffer tank, an anaerobic pond, consisting of a tank of 1200 l, and mini-ponds consisting of plastic containers (Figure 3.3 and Figure 3.4).

Wastewater collected from University Halls was poured through a raffia basket, used as rake for grit removal, in the wastewater supply tank. This tank supplied wastewater to the anaerobic pond through a pipe provided with a valve. The effluent from the anaerobic pond was collected into a buffer tank. From the buffer, the desired volume of water was transferred into the different experimental mini-ponds with an automatic sampler SIGMA SD 900 (Figure 3.4). The retention time in the anaerobic pond was five days. This retention was taken to comply with the retention time of the anaerobic pond of the pilot plant

described below. Anaerobic conditions were observed in the anaerobic tank, because it was closed.



**Figure 3.3 :** Layout of the experimental set up with mini-ponds



**Figure 3.4 :** A view of the experimental set up with mini-ponds

Two types of mini-ponds were used depending on the experiment when pre-treated wastewater was used as influent medium.

The first type of mini-pond (MP-1) consisted of plastic containers of 52 cm of length, 42.5 cm of width and 35.5 cm depth, filled with 50 l of anaerobically treated wastewater (effluent of from the anaerobic pond). The retention time for these ponds was 21 days to comply with the retention of the pilot system.

The second type of mini-pond (MP-2) was plastic containers of dimensions 50 cm × 30 cm × 20 cm. These mini ponds were filled with 20 l of the anaerobically treated wastewater with the retention time of fifteen days.

- *The set-up with mini-ponds with artificial medium as influent*

The set-up, when using an artificial prepared medium as influent, consisted of a one metre cube shaped tank, an automatic sampler type SIGMA SD 900 and the mini-ponds (Figure 3.5). The culture medium was prepared in the cubic tank. The automatic sample allowed for the homogenisation of the solution and its sampling into the mini-ponds. 20 l of the prepared solution was transferred to each mini-pond. This third type of mini-ponds (MP- 3) was monitored for a retention time of fifteen days.



**Figure 3. 5 :** *Experimental set-up with mini-ponds with artificial medium as influent*

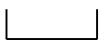
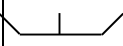
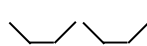
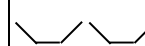
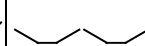
### 3.3.2. Pilot plant

Some experiments were conducted in a full scale pilot plant constructed on the University Campus of Abomey-Calavi, Benin in a tropical climate. The system was to treat the wastewater from the University halls. The generated wastewater was a typical domestic wastewater (Table 3.1). The wastewater was collected and transported from the halls to the treatment plant in a small diameter sewerage system of about 600 m of length.

The experimental system consisted of two identical continuous flow channels working in parallel, receiving the effluent of an anaerobic pond (which is considered here as the influent of the channels). A grit chamber was placed ahead of the anaerobic pond. Each channel was composed of four cement lined ponds built with reinforced concrete walls to ensure water tightness and to prevent seepage water losses. The two channels were operated under similar conditions. The dimensions of the ponds are summarised in Table 3.2. The general arrangement of each channel is shown in Figure 3.6.

The design inflow for the system was 6 m<sup>3</sup>/day. The design BOD<sub>5</sub> and COD were respectively 330 mg and 790 mg /l.

**Table 3. 2 : Pilot Plant Pond dimensions**

	Anaerobic	Ponds 1	Ponds 2	Ponds 3	Ponds 4
Shape					
Volume (m <sup>3</sup> )	23.10	34.29	25.26	25.26	42.86
Water depth (m)	2.10	1.50	1.00	1.00	1.50
Mid-depth Plan Area (m <sup>2</sup> )	13.75	22.86	25.26	25.26	28.57
Mid-depth Length (m)	5.00	7.62	6.31	6.31	4.76
Mid-depth width (m)	2.20	3.00	4.00	4.00	6.00
Total depth	2.80	2.00	1.50	1.50	2.00
Side slope n	0.00	1.00	1.00	1.00	1.50
Surface area(m <sup>2</sup> )	13.75	55.65	49.89	49.89	82.99
Top Length (m)	5.00	10.10	8.30	8.30	8.50
Top depth (m)	2.20	2 x 2.25	2 x3,00	2 x3,00	2 x4,90

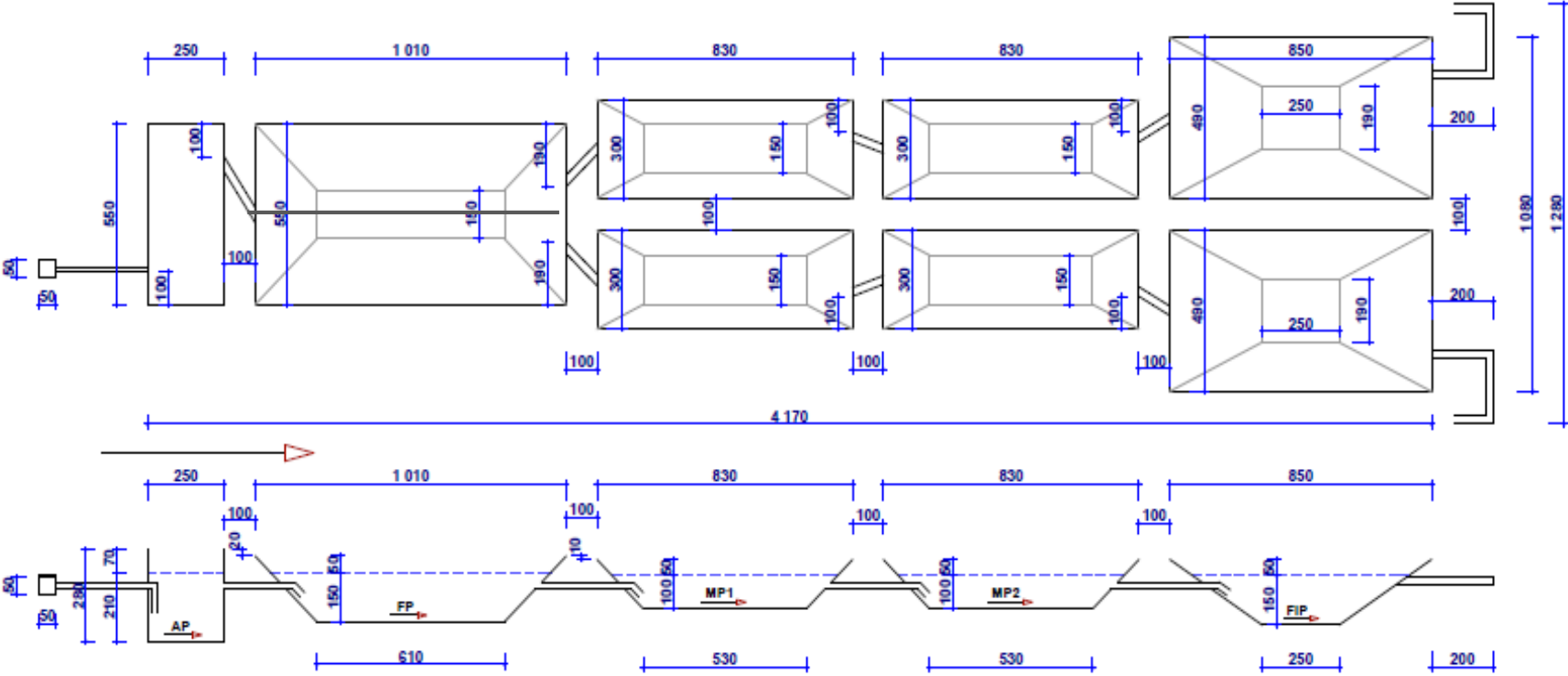


Figure 3. 6 : General arrangement of each channel of the system

Four ponds of one channel were algae based waste stabilization ponds (WSP). The first two ponds of the second line were covered by water hyacinth and the last two ponds of this channel covered with duckweed. Fish were grown in the fourth pond of each channel. The effluent of the system was intended to be used for crop farming (Figure 2.7).

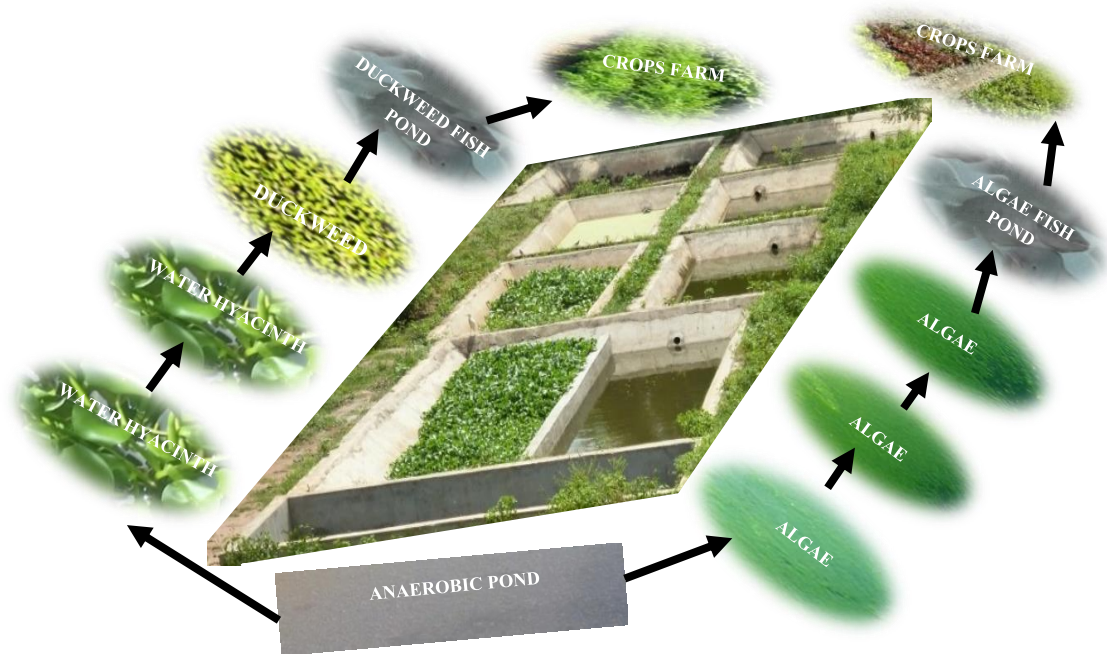


Figure 3. 7 : Pilot Plant Set-up

### 3.4 Operation

#### 3.4.1. Environmental conditions

All the works were performed in the environmental conditions of the study site which has a tropical climate condition.

Apart from part of the experiment to study the effect of light on the system, where some of the mini-ponds were placed in a closed room, all the experiments were carried outdoors under the natural environmental conditions of the study sites.

### **3.4.2. Mini-ponds operation**

The mini-ponds were operated under batch flow conditions for all the experiments in which they were used. The influent medium used was not replaced during the whole experimental period. The plants were left to grow in the ponds for the retention time or until complete wilting, if it occurred before the end of this experimental period.

The retention times selected depended on the experiment being conducted and the conclusion from relevant preceding experiments. Two retention times were used: 21 days for MP-1 and 15 days for MP-2 and MP-3.

The retention time for MP-1 was 21 days to comply with the retention of the pilot system and to optimize the results.

The retention time of 15 days for MP-2 and MP-3 was chosen by referring not only to the results of our studies with MP-1 and some literature indicating that 15 days retention is necessary for optimal reduction of organic pollutants from wastewater (Koné 2002; EFFEBI 2009), but also studies addressing heavy metal removal by water hyacinth (Jayaweera et al. 2007; Gakwavu et al. 2012).

The behaviour of the plants depends on their immediate previous history (Ashby and Oxley 1935). To avoid systematic errors related to the use of populations of different history, the experiments for each study were carried out simultaneously in parallel in different mini-ponds under the same conditions with plants from the same source.

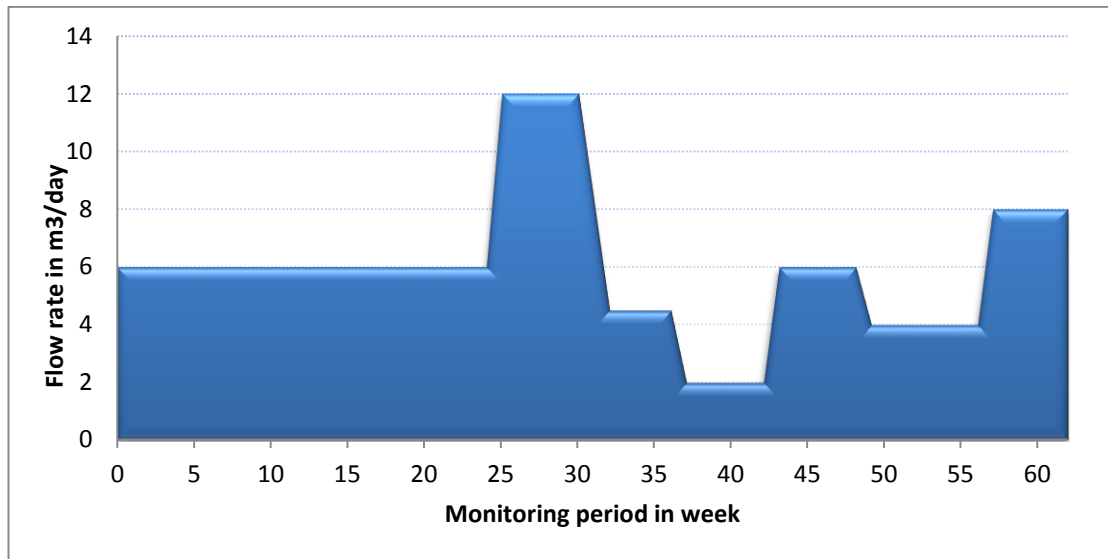
### **3.4.3. Pilot plant operation**

The pilot plant was designed to operate under continuous flow. A pre-defined volume of wastewater, corresponding to a pre-set inflow, was pumped into the anaerobic pond in every day. When run under the design hydraulic flow rate of 6 m<sup>3</sup>/day, the retention for the anaerobic pond was five days and that for each channel was twenty days.

DWPs were initially seeded with *Spirodela polyrrhiza*. Later on, a mixed culture of *Spirodela polyrrhiza* and *Lemna minor* was developed in the duckweed ponds. Also the presence of a very few plants of *Spirodela polyrrhiza* was also observed in the WHPs. Probably, seeds were brought by birds from small pilot culturing pond nearby the site and also from pond to pond. The outlets of the DWPs were covered by nets to prevent the plants from flowing out.

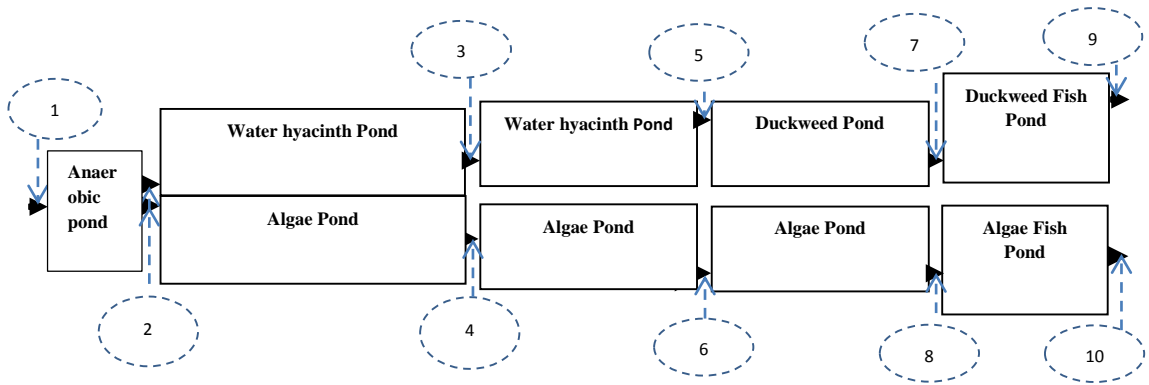
- *General monitoring frequencies and laboratory analyses*

The pilot plant was monitored for 62 weeks. The system was fed at different flow rates with different qualities of wastewater over the total experimental period. The flow rates checked were 6 m<sup>3</sup>/day; 12 m<sup>3</sup>/day; 4.5 m<sup>3</sup>/day; 2 m<sup>3</sup>/day; 4 m<sup>3</sup>/day and 8 m<sup>3</sup>/day. The total experimental period was divided into seven sub-periods where the experimented flow rates were as shown in Figure 3.8.



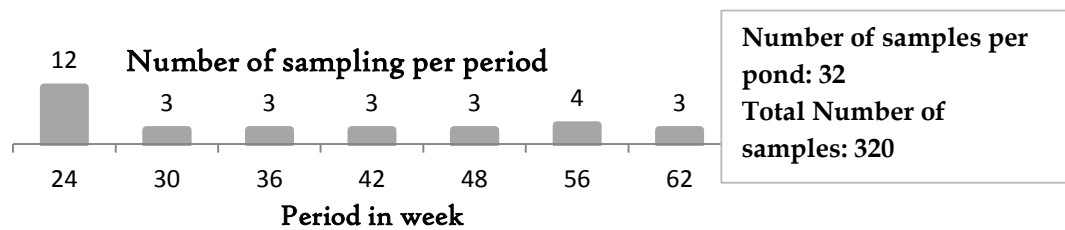
**Figure 3. 8 :** Total flow rate of the system over the experimental period

On-site measurements were conducted and samples were taken regularly at 10 different points in the system (Figure 3.9) for the determination of the parameters for performance evaluation.



**Figure 3. 9 :** Sampling points in the system

The measured parameters for performance evaluation were chemical oxygen demand (COD), total suspended solids (TSS), biochemical oxygen demand (BOD<sub>5</sub>), orthophosphate, nitrate, nitrite, total organic nitrogen (TN), total Kjeldahl nitrogen (TKN) and fecal coliforms (CF). They were measured on liquid samples collected on average of twice a month basis as presented Figure 3.10 .



**Figure 3. 10 :** Sampling for organic load, nutrient and faecal coliforms content

Additional parameters such as dissolved oxygen (DO), temperature, pH, conductivity ( $\chi$ ) and total dissolved solids (TDS) were measured in situ on average of twice a week basis.

- **Fish culturing experiment**

After 12 weeks of experiment, four fish of approximately five to six cm in length were introduced in the last pond of each channel. The growth of the fish was planned to be determined by counting them and measuring their weight. However, a more consistent approach to catching all the fish is necessary to complete these measures satisfactory.

- ***Intensive environmental parameter monitoring***

During the first period, where influent flow rate was 6 m<sup>3</sup>/day, environmental conditions (temperature, pH, conductivity, TDS, E<sub>H</sub>) in the influent and ponds of the different channels were monitored intensively 3 times a day, 5 days a week for a three-week period (no measurement on Saturdays and Sundays). During data processing, thirteen days' data were validated; two days' data were rejected for diverse reasons (some measurements were omitted in the day or seemed not well reported). The measurements were taken three times a day, from 9 to 10 a.m., 1 to 2 p.m. and 5 to 6 p.m. Four measurement points were considered over the length of each pond as follows:

- Ponds 1: 1 m; 4 m; 7 m and 9 m from the inlet side
- Ponds 2, 3 and 4: 1 m; 3 m; 5.5 m and 7 m from the inlet side

- ***Pond maintenance***

Harvesting of the plants from ponds was done manually every two weeks to have the coverage around 50%.

#### **3.4.4. Plant material**

The water hyacinth clones used were collected on Lake Nokoue located in southern Benin. They were then grown for several months in a pond on the University Campus of Abomey-Calavi. Healthy plants of similar size, shape and height were washed several times using tap water. A number of these plants, depending on the experiment, were chosen and were introduced directly into the experimental pond and without a further acclimatisation.

The duckweed plants used were collected on Lake Nokoue around Sô Ava, a lakeside town of Benin. They were cultured for several months on the university campus. Some fronds on the plants were then used to seed the ponds.

### 3.4.5. Wastewater and culture medium

- *Wastewater*

The wastewater used was from University Halls which accommodated students living on-site. The wastewater generated was then typical domestic wastewater. Different qualities of wastewater were used for the studies. They may be classified in two types as high strength wastewater and medium strength wastewater. The ranges of values for BOD<sub>5</sub>, COD, PO<sub>4</sub><sup>3-</sup>, NO<sub>3</sub>-N, NH<sub>4</sub>-N, temperature, DO, pH for these two types of wastewater are summarised in Table 3.4. The wastewater, to be treated by natural systems such as ponds, has to be biodegradable. The ratio COD/BOD<sub>5</sub> is the measure of the level of biodegradability of wastewater. The biodegradability of the wastewater is favourable when the ratio COD/BOD<sub>5</sub> is less than 2.5 (Loehr 1977) .

**Table 3. 3 :** *Typical composition of wastewater with different strength*

Contaminants	Units	Strength of the wastewater	
		Medium	High
Solids, total (TS)	mg/l	720	1200
Dissolved, total (TDS)	mg/l	500	850
Suspended Solids	mg/l	220	350
Biochemical Oxygen Demand 5-days, 20°C, (BOD <sup>5</sup> ,20°C)	mg/l	220	400
Chemical Oxygen Demand (COD)	mg/l	500	1000
Nitrogen (total as N)	mg/l	40	85
Phosphorus (total as P)	mg/l	8	15
Total Coliform	no/100 ml	10 <sup>7</sup> -10 <sup>8</sup>	10 <sup>8</sup> -10 <sup>9</sup>

<sup>a</sup> Values should be increased by the amount present in domestic water supply (Metcalf and Eddy 2003).

- **Artificial medium**

Several nutrient solutions have been used by authors for culturing macrophytes. Among those media are the Hillman solution, the Hunter medium (Landolt and Kandler 1987; Al-Nozaily 2001), 10 % Hunter (Vermaat and Khalid Hanif 1998), and the Jacob Hoagland solution (Kittiwongwattana and Vuttipongchaikij 2013).

In the present study, a synthetic Hillman solution (Table 3.4) was used as culture medium.

**Table 3. 4 : Composition of Hillman solution prepared for the experiment (Landolt, 1987)**

Substances	Concentration (mg/l)
KNO <sub>3</sub>	1515
KH <sub>2</sub> PO <sub>4</sub>	680
Ca(NO <sub>3</sub> ) <sub>2</sub> , 4H <sub>2</sub> O	1180
FeSO <sub>4</sub> , 7H <sub>2</sub> O	5,4
C <sub>4</sub> H <sub>6</sub> O <sub>6</sub>	3
MnCl <sub>2</sub> , 4H <sub>2</sub> O	3,6
ZnSO <sub>4</sub> , 7H <sub>2</sub> O	0,2
H <sub>3</sub> BO <sub>3</sub>	3
CuSO <sub>4</sub> , 5H <sub>2</sub> O	0,08
Na <sub>2</sub> , Salt of EDTA	100

The solution was prepared in the laboratory using distilled and homogenised water and a magnetic agitator. For the sake of the experiment, four hundred litres of the nutrient solution were prepared.

### **3.5 Risk assessment and safe system of work**

Each work activity contains procedures requiring a specific scheme of work. Before any activity is undertaken, it is important that engineering controls are in operation, that protective equipment requirements are met and the safe system of work and personal hygiene measures are planned and then followed.

Wastewater contains pathogenic bacteria and therefore it poses a threat to public health, to the environment and to the persons (workers) involved in the project. Mitigation measures adopted included vaccination against meningitis, tuberculosis and cholera for all the persons involved in the field and laboratory works. To avoid contact with the wastewater, plastic boots, gloves and masks for nose covering were provided. The wearing of protective clothes, mask and gloves during sampling, plants harvesting and sludge removal was mandatory.

The sludge removed and plants harvested from ponds were directed to a sludge drying bed on the composting site.

As additional security measure, to make sure nobody tampered with the pilot system, a guard was put in place to watch over the systems.

This risk assessment was completed, but no hazardous substances were used onsite in the experiments. General laboratory measures were followed for the laboratory analyses.

## 3.6 Analytical methods

### 3.6.1. Pilot plant inflow measurement

The influent wastewater flow rate was determined by measuring the daily volume pumped into the system. The total daily volume, based on the required flow rate, was divided into several elementary volumes  $V_i$  to avoid pumping the whole volume at once in an hour of the day. Each elementary volume  $V_i$  was pumped at once in the anaerobic pond. The anaerobic pond was graduated to allow for the volume determination. During the pumping of each volume  $V_i$ , the outlet of the anaerobic pond was closed to allow for reading on the graduation. After the reading, the wastewater was allowed to flow into the channels. The daily mean flow rate was determined by the following formula:

$$Q = \frac{\sum_{i=1}^n V_i}{t} \quad (3.1)$$

Where  $V_i$  is the elementary volume in litre;  $n$ , the number of pumping in a day;  $t$  = 24 h

### 3.6.2. Organic loading rate determination

The total organic loading rate was calculated by (Mara 1987):

$$\lambda_v = 1000 C_{BODin} Q \quad (3.2)$$

Where,  $C_{DBOin}$  is the influent  $BOD_5$  (or COD) into the system in  $mg O_2/l$ ;

Q the influent flow rate in m<sup>3</sup>/day

$\lambda_v$  is expressed in kg/day.

### 3.6.3. Plant growth measurement

The growth rates were measured by counting separately the number of plants and/or weighing the wet biomass at the beginning and the end of the experimental period. The wet mass was measured by removing the excess water by placing and rolling the plants cautiously between absorbent paper tissues and by weighing the biomass immediately after that. Knowing the biomass weight, the plant relative growth rate (RGR) was calculated by equations below (McLay 1976; Al-Nozaily 2001; Hounkpè *et al.* 2013):

$$RGR = \frac{[\ln(n_f / n_i)]}{t} \quad (3.3)$$

or

$$RGR = \frac{[\ln(m_f / m_i)]}{t} \quad (3.4)$$

Where  $m_i$  and  $m_f$  (or  $n_i$  and  $n_f$ ) are respectively the initial and the final wet weight (or number) of plants at the start and the end of the experimental period and  $t$  is the number of days between two weighings or countings.

The biomass doubling time can be calculated by equation 3.5:

$$Doubling\ Time = \frac{[\ln(2)]}{RGR} \quad (3.5)$$

### 3.6.4. Environmental parameter measurement

Environmental parameters such as pH, temperature, conductivity ( $\chi$ ), dissolved oxygen (DO), light intensity and Total Dissolved Solids (TDS) were measured on site.

pH and temperature were measured with the pH-meter pH 3110 SET 3 (WTW) of  $\pm 0,01$ pH of accuracy in accordance with the standard *NF T 90-008*.

The light intensity was measured with *the Digital Lux Meter Model: LX1330B*.

The electrical conductivity, in  $\mu\text{S}/\text{cm}$ , was measured with a conductivity-meter pH/EC/TDS Waterproof Family in accordance with the standard *NF EN 27888 class index T 90-031*. The salinity was deducted from the measured conductivity in accordance with the standard *NF T 90-111* using equation 3.6 (Thomas 1995).

$$\text{Salinity} \left( \text{in } \frac{\text{mg}}{\text{L}} \right) = K * \text{conductivity} (\mu\text{S}/\text{cm}) \quad (3.6)$$

K is a constant depending on the conductivity.

The dissolved oxygen was measured with the oxymeter Oxi 730 in accordance with *NFT 90-106 standard*.

### 3.6.5. Suspended solids, organic matter and nutrients determination

During the experiments, water samples were collected from ponds and analysed for total suspended solids (TSS), biochemical oxygen demand ( $\text{BOD}_5$ ), chemical oxygen demand (COD), ammonia-nitrogen ( $\text{NH}_4^+\text{-N}$ ), nitrate-nitrogen ( $\text{NO}_3^-\text{-N}$ ), nitrite nitrogen ( $\text{NO}_2^-\text{-N}$ ), Total Kjeldhal Nitrogen (TKN), total nitrogen, orthophosphate-phosphorus ( $\text{PO}_4^{3-}\text{-P}$ ). All of these parameters were tested using standard laboratory procedures and methods and all analyses were completed within 24 h of sample collection.

- *TSS determination*

TSS, expressed in  $\text{mg}/\text{l}$ , was measured by filtration (for water with a low load) or by the centrifuge method according to *NF EN 872 standard*, class T 90-105-1 and class T 90-105-2 standard, respectively (Rejsek 2002). A filter device Sartorius Stedium, an oven DRY – Line VWR, a centrifuge device VWR Compact Star CS 4 and a pump Vaccum Gas Pump, VRW were used for the measurements.

- *COD determination*

COD is a representative quality parameter for a normalised effluent according to the standard standard ISO 15705. COD, expressed in  $\text{mg O}_2/\text{l}$ , is the quantity of potassium dichromate consumed by the pollutants present, when the sample of water is treated by this oxidant in standard conditions (Rejsek 2002). COD was

determined by the volumetric method using the Hach Tubetests System and a spectrophotometer DR 2800 according to the standard standard NFT 90-101 (Rejsek 2002). Hach Tubetests were integrated with a Hach COD reactor with 25 holes. In this method, the water sample is oxidized by digesting it in the COD reactor in a sealed reaction tube with sulphuric acid and potassium dichromate in the presence of a silver sulphate catalyst.

- ***BOD<sub>5</sub> determination***

BOD is a measure of oxidizable substances in a water sample. It is determined by measuring the concentration of dissolved oxygen consumed during the incubation of the water sample in an OxiTop in a constant temperature chamber. The chamber is thermostatically controlled to maintain a constant temperature and exclude all light to prevent the possibility of photosynthetic production of oxygen during incubation (standard standard NF T 90-103) (Rejsek 2002). The incubation period for BOD<sub>5</sub> is five days at 20 degrees Celsius. The measurement principle of the DO consumed is based on measuring pressure differences estimated by piezoresistive electronic pressure sensors.

- ***Nutrient determination***

TKN is the sum of the total organic and ammonia nitrogen (Rodier *et al.* 2009). The measurement principle was the mineralisation, in a nitrogen digestion apparatus K-424, by selenium in presence of sulphuric acid at 400°C in accordance with the standard NF EN 25663 class T 90-110. The quantity of ammonia formed after digestion and distillation was determined by acidimetric titration (standard NFT 90-015) (Rejsek 2002).

Nitrate and nitrite were measured by the method of molecular absorption using the spectrophotometer DR 2800 according to standard FD T 90-045 and the standard NF EN 2677 class T 90-013, respectively. Orthophosphate was also determined by the same method NF EN ISO 15587-1 (Rejsek 2002).

Ammonia nitrogen was determined by a colorimetric method with indophenol blue (NF T 90-015) (Rejsek 2002).

### 3.6.6. Microbial quality determination

The coliform group of organisms is used as indicator of bacterial pathogenic organisms for water quality determination. The faecal bacteria include the genera *Escherichia* and *Aerobacter* (Greenberg et al., 2003; Awuah, Mara 76). The method used for faecal coliforms (FC) examination was the count in a solid medium by agar incorporation. They were counted using the Rapid-E Coli medium (24 h at 44 °C) according to the standard NF-08-05. The results were expressed in number of FC per 100 ml.

### 3.6.7. Metal pollutant determination

The metals checked in this study were iron, zinc and copper.

- *Metal concentration in solution*

The concentrations of iron, zinc and copper in solution, expressed in mg/l, were determined using the spectrophotometer HACH DR-2800 by the standardised method USEPA 8147, 8009 and 8506 respectively. The principle is based on formation of coloration through the reaction of the metal with the appropriate reagent. The intensity of coloration is proportional to the amount of the metal in the solution. This colour absorbs part of the light radiation of the spectrophotometer according to Beer-Lambert Law (Rejsek 2002; Rodier *et al.* 2009).

- *Metal concentration in plant tissues*

To determine the metal concentration in plant tissues in the study, the submerged part of the sample of plant was dried at 105°C up to a constant mass. Tissue samples were ashed in a furnace at 450 °C for 5 h. The ashed samples were then mineralised and analysed for metal content in the plants using an atomic absorption spectrophotometer. The accumulation of the metals by the plants was then calculated. The metal content of the top part (out of water) was not determined because studies have concluded that the major part (over 90%) of metal adsorbed by water hyacinth is accumulated in the submerged part of the plants (Hasan et al. 2007).

## **3.7 Results analysis method**

The results analysis method was based on the statistical analysis of the collected data. Data analysis was conducted based on average value, standard deviation, the variance analysis and a principal components analysis. Histograms, coordinate lines, error bars and boxplots were used for data visualisation.

### **3.7.1. Analysis of variance: ANOVA**

Analysis of variance (ANOVA) is a particular form of statistical hypothesis testing used as an exploratory tool to explain observations. A statistical hypothesis test is a method of making decisions using data. A test result is statistically significant when a probability (p-value) is less than a significance level (threshold), assuming the truth of the null hypothesis. The XLSTAT statistical package was used to for this analysis.

### **3.7.2. Principal components analysis (PCA)**

The principal components analysis is a statistical tool to analyse the relationship between collected data.

The principal component analysis (PCA), invented in 1901 by Karl Pearson, has a purely descriptive objective. It is based on the calculation of averages, variances and correlation coefficients. It is intended to obtain a subspace of independent variables (principal components) giving the best possible viewing of our cloud of points from a linear combination of the initial variables. Data analysis is essentially to establish the relationship between the observations, between variables and between observations and variables.

For a set of  $n$  observations over a number of  $p$  parameters, the PCA consists of the projection of the points on a straight line, a plane or a sub-space of dimension  $s$  ( $s \leq p$ ) giving the best visualisation possible of the cloud in order to optimise a criteria. This sub-space is defined by an orthonormal system of lines which are the principal components, with the Eigenvectors as their directing vectors.

The matrix of the mean centred values of the data  $X_{np}$  is given by:

$$X_{np} = \begin{pmatrix} x_{11} & \cdots & x_{1p} \\ \vdots & \ddots & \vdots \\ x_{n1} & \cdots & x_{np} \end{pmatrix} ;$$

The main vectors, that are the Eigenvectors, are given by:

$$(X_{np}^T X_{np} - \lambda I_m) \cdot \mathbf{u} = \mathbf{0} \quad (3.7)$$

Where  $X_{np}^T$  is the transpose of  $X_{np}$  and  $I_m$  the unit matrix;

The score of the variables or observations are their projections on the main vectors. The score is given by:

$$\text{Variables: } C_V = X_{np}^T V = U \Lambda^{1/2} \quad (3.8)$$

$$\text{Observation: } C_o = X_{np} U = V \Lambda^{1/2} \quad (3.9)$$

$V$  is the matrix having the  $p$  Eigenvectors of  $XX^T$  placed in the columns,  $U$  the matrix of the  $p$  eigenvalues  $X^T X$  in row and  $\Lambda$ , the diagonal matrix having the  $p$  Eigenvalues in column.

The quantification of the importance of a variable (or observation), or its contribution, to the definition of an Eigenvector is given:

$$\text{Ctr} (S_i, CP_k) = \frac{(\text{score of } S_i \text{ according to } CP_k)^2}{\sum_j (\text{Score of } S_j \text{ according to } CP_k)^2} \quad (3.10)$$

The PCA used in this study is the PCA of the Pearson correlation matrix. The PCA of correlations is the most common PCA. It has the advantage of using variables without unit and leads to results that are independent of the original units of the variables. Thus it allowed the analysis of variables of very different order of magnitude.

The XLSTAT software add-in Excel 2010 was used for calculation of the "Pearson" type PCA. The "absolute" criterion called Kaiser Criterion, which is to retain only the axes with Eigenvalues greater than 1, was used in this case to choose the two-dimensional projection used to create a binary diagram. The

axes, which are the principal components, are represented by F1, F2 , ..., FP in the software. The projection plan is the factorial plan. The analysis of the data in a factorial plan is acceptable when this plan explains over 70% of the total variance (Cooley and Lohnes 1971; Lebart *et al.* 1984).

### **3.7.3. Boxplots**

The boxplots display variation in samples of a statistical population which allows for the analysis of the relationship between qualitative and quantitative variables. A boxplot gives a simple and practical summary of a distribution by the measurements of the minimum, the first quartile q (25%), the median, the third quartile q (75%) and the maximum. They are useful for comparing several groups of observations like in the present case.

# 4

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## Influence of solar radiation on water hyacinth ponds performance using high strength sewage

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### 4.1 Introduction

The efficiency of water hyacinth in removing pollutant from wastewater, in tropical areas, has been proved by several researchers. However, the treatment performance of water hyacinth is highly related to their growth and multiplication (Kengne *et al.* 2000). Unfortunately, as all biological process, the growth of water hyacinth, and then its wastewater purification potential are influenced by a number of factors, such as nutrient availability, pH, light intensity, temperature, the dissolved oxygen content and the salinity of the water (Gopal 1987; Center *et al.* 2002; Williams *et al.* 2005; Coetzee *et al.* 2009; Yi *et al.* 2009).

In a wastewater treatment plant it is a fact that there may be a change in light intensity. A search of the literature for evidence concerning the effects of the fluctuation of the light intensity, as related to the position of ponds relative to sunshine, on the wastewater purification performances of water hyacinth ponds showed that, no investigation has not been carried out to observe the effect.

The study reported in this chapter aims to demonstrate that changes in light intensity can affect the pollutant removal performance of water hyacinth ponds and their biomass production rate.

The objective of this chapter is to investigate the influence of direct sunshine on water hyacinth and their wastewater purification performance. This investigation is necessary for the determination of the optimum position of water hyacinth ponds with regards to sun exposure for their use in water treatment and biomass production.

## **4.2 Materials and methods overview**

The first type of mini-ponds (MP-1) was used in this experiment. Anaerobically treated high strength wastewater was used as culture medium in the mini-ponds.

The experiment was carried out with nine MP-1 filled with 50 l of anaerobically treated wastewater and retention time of 21 days as described in Chapter 3. Four green plants (with mean fresh weight  $280 \pm 4$  g) were inserted into each container. The first three containers (MP-1room) were placed in a closed room; the next three under shade (MP-1shade), but receiving sunlight and the last three ponds were placed in full sun light (MP-1sun).

The MP-1sun exposed directly under sun outdoors were sometimes stored under shade at night and taken out early every morning to avoid dilution by rainwater, which would affect the effluent quality and lead to an error in the estimation of the removal rate of pollutants.

The environmental parameters pH, the redox potential Eh, conductivity  $\chi$ , dissolved oxygen DO, temperature T and light intensity were measured every day. The day where growth started in ponds was recorded. The number of plants was counted in each pond at the beginning, the seventh, the fourteenth and the 21<sup>st</sup> day. Samples were taken at the same days for the measurement of total suspended solids (TSS), COD, BOD<sub>5</sub>, nitrate, nitrite, orthophosphates and total Kjeldahl nitrate TKN. The measurement TSS, COD, BOD<sub>5</sub> of MP-1room was stopped on the seventh day after the complete degeneration of plants in these ponds. The plant fresh weights were measured at the beginning and the

end of the experiment. The light intensity was measured 3 times a day (9 a.m., 12 p.m. and 15 p.m.) every day.

### 4.3 Results and discussion

#### 4.3.1. Performance of anaerobic treatment

The BOD<sub>5</sub> and COD characteristics of the raw influent wastewater showed that it was a high strength domestic wastewater (Metcalf and Eddy 2003). The raw sewage had a BOD<sub>5</sub> varying between 445 ± 7 O<sub>2</sub>/L and COD between 975 ± 15 mg O<sub>2</sub>/L. The COD / BOD<sub>5</sub> of the influent was very close to 2 indicating that the influent was biodegradable and can be treated by a pond system..

**Table 4. 1 : Raw wastewater and anaerobic pond effluent**

Parameters	Unit	Raw sewage	Effluent anaerobic pond	Removal rate %
Temperature	°C	26.3 ± 0.0	26.3 ± 0.0	
pH	U pH	6.83 ± 0.02	6.789 ± 0.001	
e <sub>H</sub>	mV	0.7 ± 0.2	19 ± 0.1	
r <sub>H</sub>		13.67 ± 0.03	13.64 ± 0.01	
χ	μS/cm	1526 ± 12	1655 ± 5	-
Turbidity	NTU	297.3 ± 2.0	151.3 ± 1.3	49%
COD	mg /l	975 ± 12	297 ± 6	70%
BOD <sub>5</sub>	mg /l	445 ± 7	155 ± 3	65%
TSS	mg/l	360 ± 4	120 ± 1	67%
TKN	mg/l	108 ± 3	84 ± 0.5	22%
N-NO <sub>3</sub> <sup>-</sup>	mg/l	0.04 ± 0.01	0.01 ± 0.0	75%
N-NO <sub>2</sub> <sup>-</sup>	mg/l	0.001 ± 0.000	0.022 ± 0.00	-
P-PO <sub>4</sub> <sup>3-</sup>	mg/l	20.2 ± 0.5	14.7 ± 0.1	27%

A comparison between the physio-chemical parameters of the raw wastewater and the reported quality of wastewater from University of Abomey-Calavi's Halls showed conformity with reported values for these wastewaters (Houngpè *et al.* 2013)

Temperature, pH and  $r_H$  of the raw wastewater and anaerobic pond were almost the same. The observed temperature was optimum for anaerobic degradation in the pond (Gloyna 1972; Mara *et al.* 1992) (Effebi 2009). The average COD, BOD<sub>5</sub> and TSS concentration of the raw wastewater was reduced by 70%, 65% and 67% respectively, in the anaerobic pond as recommended by several authors for primary treatment (Koné 2002; Effebi 2009). The observed organic load removed complied with the reported BOD<sub>5</sub> removal which ranged between 40 and 80% and even more depending on the temperature (Gloyna 1972; Mara *et al.* 1992). Nutrient removal in this pond was low. 22% of TNK removal was observed while phosphate removal of 27% was achieved with the pre-treatment. The quality of influents and effluents from the anaerobic pond is shown in Table 4.1. A reduction in nitrite concentration from 0.04 mg/L to 0.01 mg/L and an increase in nitrate from 0.001 mg/L to 0.022 mg/L were observed. This increase in the nitrate concentration may be associated with the oxidation of the nitrite into nitrate in this pond.

#### 4.3.2. Environmental parameters in water hyacinth ponds

*Light intensity:* The light intensity observed in MP-1room was very low (about 33 lux) throughout the experimental period. This value is negligible compared to that of MP-1shade and MP-1sun (Figure 4.1).

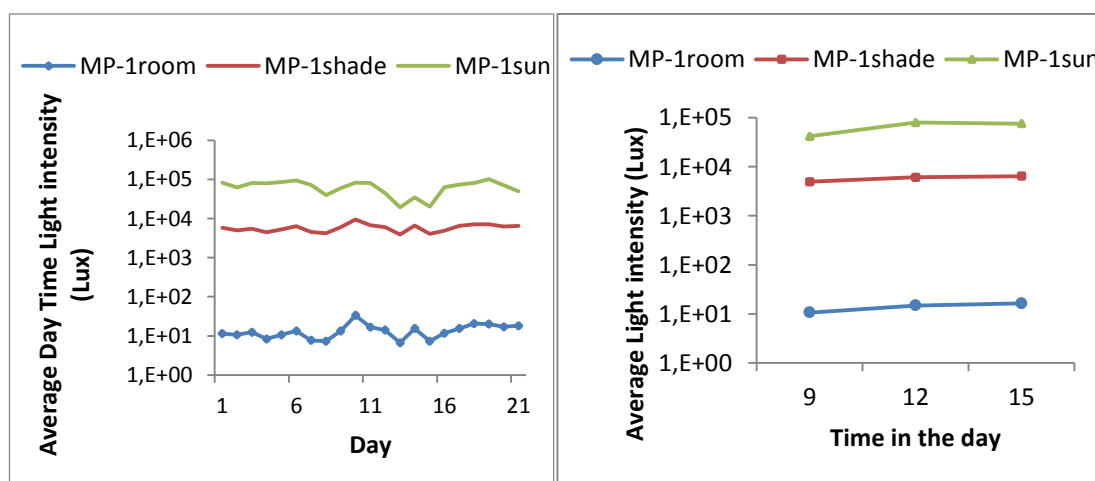
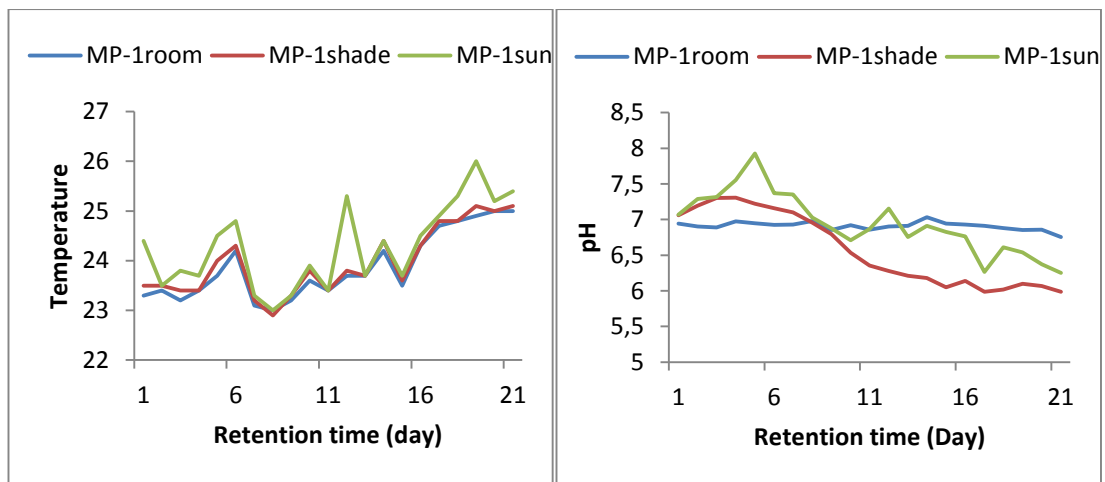


Figure 4. 1 : Changes in light intensity in ponds

The changes in light intensity in MP-1shade and MP-1sun followed the same pattern. However, the variations amplitudes of the average light intensity in MP-1sun during the day times as well as over the experimental period were more pronounced than that in MP-1shade.

The light intensity values in ponds were roughly stable. In addition, the values of light intensity of MP-1sun were always fare higher than that in MP-1shade.

The light intensity values in MP-1room ( $13.9 \pm 5.9$  Lux) were smaller than that required for photosynthesis activities for water hyacinth and resulting in the photoinhibition and then to the complete degeneration of the plants (Walker 2009).



**Figure 4. 2 :** Evolution of the temperature **Figure 4. 3 :** Evolution of the pH

The pond water temperature variations followed the same pattern, even though the water temperatures in MP-1sun were always higher than those in the other ponds (Figure 4.2). This difference of temperature in ponds was related to the direct effect of sunshine on the MP-1sun. The pattern of the temperature curves during the experimental period was due to the weather conditions.

The pH values for the MP-1room were roughly stable throughout the experimental period, taking values between 6.945 and 6.754. In MP-1shade and MP-1sun, a gradual increase from the beginning of the experiment till the fifth

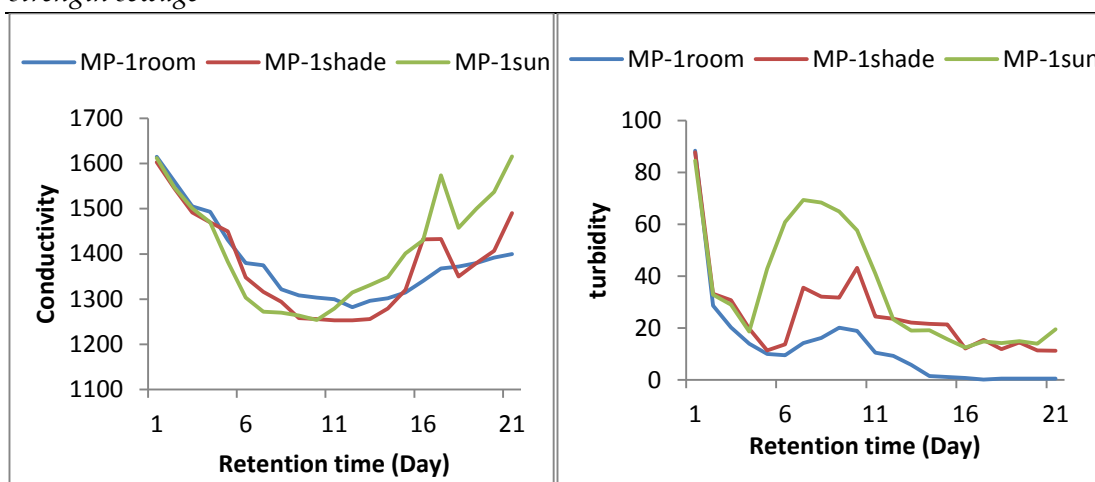
day was observed and then, the pH started decreasing (Figure 4.3). The maximum value of pH observed was 7.9 in MP-1sun.

The increase in pH at the beginning of the experiment may be associated to the reactions occurring in the medium for the adaptation of the plants and the growth of algae observed in MP-1shade and MP-1sun during this adaptation phase for the water hyacinth. The presence of algae was more pronounced in MP-1sun, where the pH values reached their maximum of 7.9. The absence of algae in MP-1room may be explained by the low light intensity which did not allow their growth.

The progressive decrease in pH observed in MP-1shade and MP-1sun after the fifth day may be explained by the release of  $H^+$  in solution by water hyacinth in compensation of nutrients consumed. It confirmed the statement of Kim and Kim that the root surface of the water hyacinth has a negative electrostatic charge (Kim and Kim 2000). Therefore, a decrease in pH may result in neutralization of the electrostatic charges through the release of  $H^+$  ions and promote diffusion of the particles in the plant roots, and therefore their retention. Likewise, after adsorption of specific nutrients such as compounds of ammonia, water hyacinth releases  $H^+$  ions to compensate (Bendada 2005).

The changes in pH in MP-1shade were smoother than that of MP-1sun. It may be related to the low fluctuation in light intensity in these ponds.

Conductivities values in all the ponds (1,2,3) dropped from the first day of insertion of water hyacinth clones in ponds up to a minimum value after which they started increasing again up to the end of the experiment (Figure 4.4). The change in conductivity values seemed to not be directly related to the presence of the water hyacinth in ponds as, even after the degeneration of plants in MP-1room the same pattern of variation was observed.



**Figure 4. 4 :** Evolution of the Conductivity **Figure 4. 5 :** Evolution of the turbidity

The initial turbidity of ponds influent dropped quickly after the insertion of water hyacinth in ponds (Figure 4.5). This was associated to the rapid settling of the suspended solids, as plants growth was not observed at this adaptation phase. In MP-1room, the water turbidity increased slightly from an average of 9.5NTU at the sixth day to 20.1 NTU the ninth day, and then dropped rapidly to reach values close to zero at the end of the experiment. The increase in turbidity was due to the degeneration of plants observed in MP-1room between the fourth and seventh days, after which the rapid settling of the plants waste resulted in a drop of the turbidity. The increases in turbidity values observed in MP-1shade and MP-1sun, before the seventh day, were associated to the development of algae bloom in ponds.

### 4.3.3. Pollutant removal performance

From Figure 4.6, it can be observed that BOD<sub>5</sub> removal followed the same pattern as COD removal. This can be explained by the direct relationship between the two parameters. The COD and BOD<sub>5</sub> removal of 49% and 50%, 70 % and 66% and 54% and 58% were achieved after the seventh day, respectively in MP-1room, MP-1shade and MP-1sun.

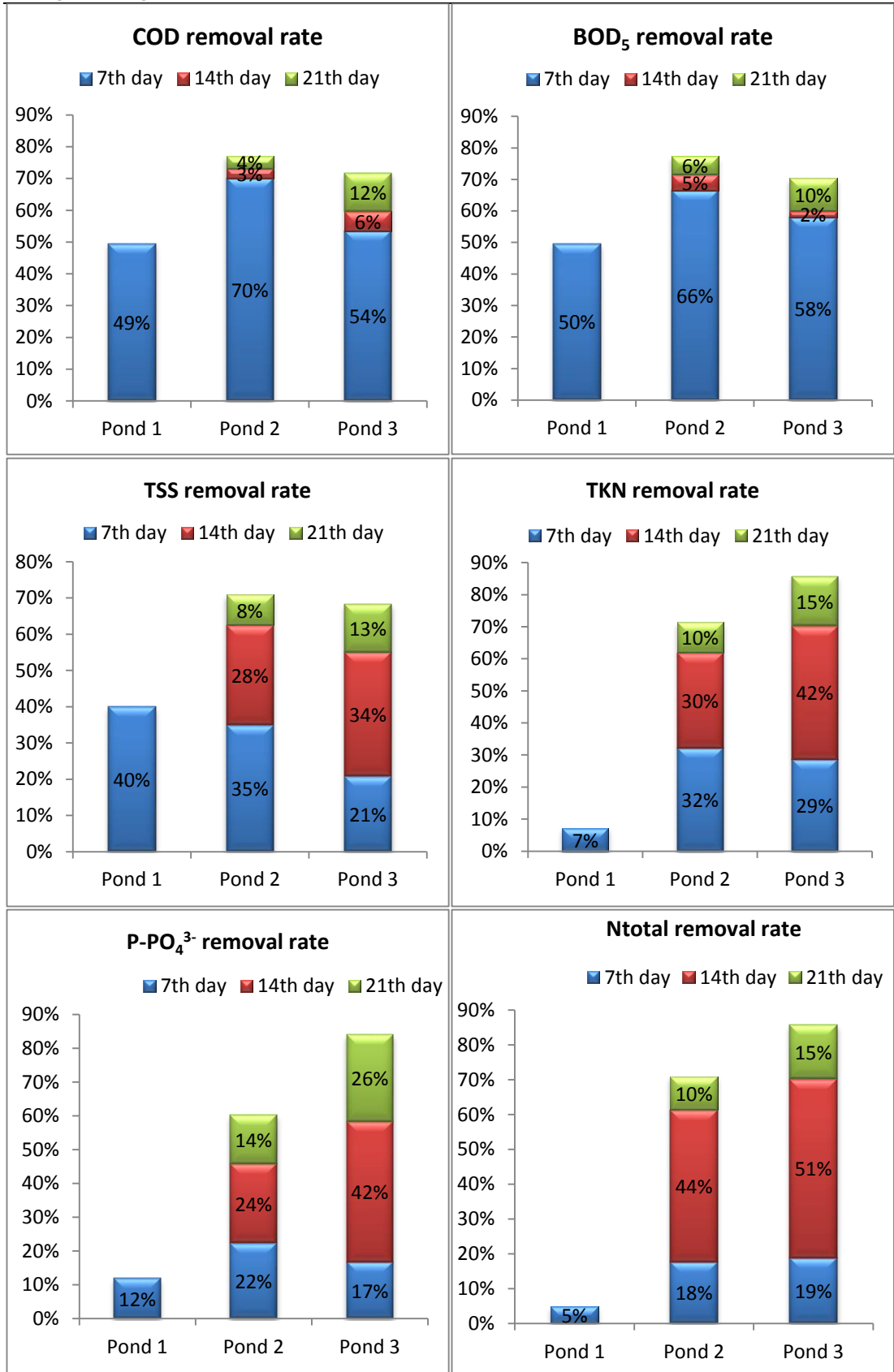


Figure 4. 6 : Organic matter and nutrients removal rate from water

At the end of the experimental period, 77% of COD and 77% of BOD<sub>5</sub> were removed in MP-1shade while 72% of COD and 70% of the BOD<sub>5</sub> removal were observed in MP-1sun. In MP-1shade more than 86% of removed organic load was achieved in the first seven days. This proportion reached 93% after 14 days retention time. These proportions of organic loads removed at the fourteenth day were respectively, 75% and 83% of the total COD and BOD<sub>5</sub> removed from MP-1sun.

The suspended solids removal seemed to be progressive (Figure 4.6). After seven days retention, the removal of TSS was 40%, 35% and 21%, respectively in MP-1room, MP-1shade and MP-1sun which represented 49% and 30% of the total TSS removed from water in MP-1shade and MP-1sun respectively, during the experimental period.

Nutrients (phosphate, TKN and total) removal in ponds followed the same pattern with the highest removed proportion observed MP-1sun from the seventh day. At the end of the 21 days, the reduction TKN, PO<sub>4</sub><sup>3-</sup>, Total Nitrogen concentrations was 71%, 60% and 71% respectively, for MP-1shade and 86%, 84% and 86% respectively, for MP-1sun (Figure 4.6).

The average organic and nutrients removal rate in MP-1shade under shade after the 21 retention time, as determined per metre square per day, was 883 g, 463 g, 582 g, 598 g and 76 g respectively for COD, BOD<sub>5</sub>, TKN Total N and PO<sub>4</sub><sup>3-</sup>. In MP-1sun, under full sun light the removal achieved per metre square per day was estimated to 711 g, 345 g, 711 g, 731 g and 114 g respectively for COD, BOD<sub>5</sub>, TKN Total N and PO<sub>4</sub><sup>3-</sup>.

#### **4.3.4. Plant growth rate**

Measurements of plant growth focused on MP-1shade and MP-1sun, as in MP-1room, at the seventh day, all the plants were dead.

From visual observation, the adaptation phase for water hyacinth plants in MP-1sun lasted longer than in MP-1shade. At the sixth day, no new plant was

observed in MP-1sun, while in MP-1shade, plant multiplication started much earlier before this sixth day. At the end of the experimental period, the plants in MP-1shade were more mature and larger than those in MP-1sun which were bright green colour with smaller size (Figure 4.7) because plant multiplication rate was higher in this pond. This was confirmed by average mass per plant which was 41.4g for MP-1shade and 38.8 g for MP-1sun.



a) In the room

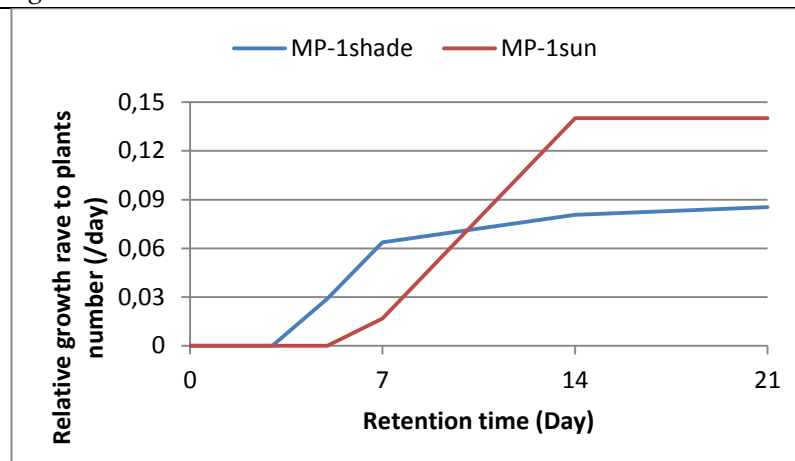
b) under shade

c) under full sunlight

**Figure 4. 7 :** *Some ponds at the end of the experimental period*

The growth pattern of plants, as measured by increase in number of clones, showed an appreciable temporal difference under shade and full sunlight over the experimental period. The growth started earlier under shade, and the relative growth rate with regard to plant number reached 0.064/day around the seventh day; this value was close to its maximum value under shade which was 0.085/day. The growth of plants was almost nil the first seven days in MP-1sun. The relative growth rate with regard to plants number was 0.017/day around the seventh day. From then it started increasing and then became stable toward the end of the experiment at the maximum value of 0.140/day (Figure 4.8). The relative growth rate in Figure 4.8 was calculated taking the end of the previous period as the beginning of the next period.

The average relative growth of plants during the 21 days, as measured by increase in fresh weight of plants was 0.052/day and 0.070/day respectively, under shade and full sunlight. These growth rates corresponded to a doubling time of 13.2 days and 9.9 days and an increase in fresh weight of 434 t/ha.year and 752 t/ha.year respectively, under shade and full sunlight. The plant average was higher under full sunlight with average sunlight of 65 955lux.



**Figure 4. 8 :** Change in relative growth rate to plants number

By taking into account the nutrient removed by other means (by reference to MP-1room) nutrient up-take by yield can be estimated at 5.4 mg/g of fresh weight (FW), 5.4 mg/g of FW and 0.8 mg/g of FW respectively for TKN Total N and  $\text{PO}_4^{3-}$  under shade and 6.5 mg/g of FW, 6.5 mg/g of FW and 1.1 mg/g of FW respectively for TKN Total N and  $\text{PO}_4^{3-}$  under full sun.

#### 4.3.5. Discussion

The change in light intensity in ponds has an appreciable effect on the growth and subsequently on ponds performance in removing organic matter and nutrients. The growth rate observed under shade was 434 t/ha.year or 119 g/m<sup>2</sup>.day and 752 t/ha.year or 206 g/m<sup>2</sup>.day was observed under full sunlight. Ho and Wong (1994) have reported a growth rate of 225 g/m<sup>2</sup>.day of water hyacinth in a secondary treatment pond where the influent BOD<sub>5</sub> was about 45mg/L in natural condition conditions with an average daily temperature of 24.5°C. The growth rate observed here was smaller. This may be due the non-renewal of the culture medium and to the fact that the adaptation period of the plants to the medium, where there was no growth, was taken into account for the determination growth rate in the present experiment. In fact, in Ho and Wong's experiment, the water hyacinth plants were already in the treatment ponds and were adapted to medium. Their experiment was under continuous

flow which allowed for renewing of the culture medium while batch flow was used here.

The adaptation period of the plants in the ponds under shade was relatively short (Figure 4.8). This may be attributed to the low variation in the light intensity during day time in MP-1shade.

In MP-1room, where there was no growth and plants died off, and no growth of algae biomass, 49% of BOD<sub>5</sub> was removed after seven days retention, essentially by settling and bacterial activities. In MP-1shade and MP-1sun 70% and 54% of BOD<sub>5</sub> removal were achieved during the same period. Then, from the total BOD<sub>5</sub> removed these seven days, 25% and 14% of the respectively from MP-1shade and MP-1sun may be accounted for the growth of the water hyacinth plants in these ponds., The remaining portion was removed by settling as in MP-1room. Similarly, with the settling and microorganism activities, 7% and 12% of TKN and phosphate respectively were removed. From the total nutrient removal from ponds, plants up-take accounted for 47% and 29% of phosphate and 78% and 75% of TKN, respectively in MP-1shade and MP-1sun during these first days. The organic matter and nutrient reduction rate in MP-1shade this period was higher than that in MP-1sun. This was due to the faster growth of plants in MP-1shade observed during this period (Figure 4.8).

From the seventh day to the end of the experimental period, the organic matter and nutrient removed from MP-1sun were higher than that of MP-1shade. This situation was reflected by plant relative growth in the ponds.

The maximum TKN, NT and PO<sub>4</sub><sup>3-</sup> removal of 711 g, 731 g and 114 g respectively, was observed in MP-1sun. The average nutrient up-take per unit gram of plants was higher under full sunlight than that under shade. The light intensity, apart from influencing the plant growth, affected the rate of nutrient up-take by plants. With higher light intensity, higher nutrient up-take rate by plants were observed which means that even with the same growth rate, the nutrient pollutant removal rate will be faster under full sunlight. So ponds

under full sunlight will require lower retention to achieve a same nutrient removal compared to a pond under shade. Also, with high nutrient up-take, the water hyacinth clones from the ponds under sun may contain higher proportion of protein per unit gram, which will make them more suitable as green fertilizer just after a sun dry of the biomass harvested.

#### **4.4 Conclusions and recommendations**

Water hyacinth ponds were used for biomass production and treatment of the effluent from anaerobic pond under different light intensity conditions. An experiment was carried out under batch flow condition with nine mini-ponds filled with 50 l of anaerobically treated wastewater seeded with water hyacinth plants. The retention time was 21 days

The anaerobic pond was able to achieve a removal of 70% of COD, 65% of BOD<sub>5</sub>, 67% TSS, 22% of TKN and 27% on the P-PO<sub>4</sub><sup>3-</sup> for a residence time of five days.

Under a restricted average day light intensity of 14 lux, no growth of plant was observed. The maximum biomass of 752 t/ha.year and nutrients up-take of 711 g/m<sup>2</sup>.day for TKN and 114 g/m<sup>2</sup>.g respectively for COD, BOD<sub>5</sub>, Total N and PO<sub>4</sub><sup>3-</sup> were obtained under full sunlight.

This experiment showed that light intensity affect biomass production and pollutant removal in water hyacinth ponds. These ponds performed better under direct sunlight. Also, the light intensity affected plant nutrient up-take rate. It was observed that the up-take of 5.4 mg/g of FW, 5.4 mg/g of FW and 0.8 mg/g of FW respectively for TKN Total N and PO<sub>4</sub><sup>3-</sup> under shade. These rates reached 6.5 mg/g of FW, 6.5 mg/g of FW and 1.1 mg/g of FW respectively for TKN Total N and PO<sub>4</sub><sup>3-</sup> under full sun.

For an optimum use of water hyacinth ponds for biomass production and wastewater treatment, ponds should be placed in areas their can receive direct sunlight.

# 5

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## ***Influence of pH on water hyacinth ponds performance using medium strength sewage***

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### **5.1 Introduction**

pH is an important environmental parameter in wastewater treatment. In macrophyte ponds, aquatic organisms and plants, which are necessary for the treatment process, are sensitive to pH changes meaning that pH monitoring or control are required for such ponds. Low pH has been found to significantly inhibit water hyacinth growth (Gopal 1987; Center *et al.* 2002; Allgayer 2006; Téllez *et al.* 2008). Similarly, Azov and Goldman (1982) have reported that high pH level is detrimental to water hyacinth growth due to deficiencies in nitrogen stripping.

Most research work on the effect of pH on water hyacinth has focused mainly on the determination of the limit pH after which plants cannot growth. The present research is focused on determining the relationship between pH and the performance of water hyacinth ponds for domestic wastewater treatment and nutrient recycling. The objectives of this chapter are to determine the effects of acidic, neutral and basic ranges of pH on water hyacinth biomass production and performance in ponds for wastewater treatment in a batch flow condition.

## 5.2 Materials and methods overview

The experiment was carried out with the mini-ponds MP-1 (see Chapter 3) using a medium strength wastewater as described in Chapter 3.

Five (05) mini ponds MP-1 (with 2 duplicate for each which made in total 15) containing anaerobically treated wastewater at different pH varying from 5 to 9 were used. The ponds occupied a total surface area of about 4 m<sup>2</sup>; the small surface covered reduced the environmental heterogeneities in the ponds. The cultures were started with six (06) water hyacinth healthy plants. The total experimental period was 21 days.

In previous studies on the effect of the pH on other plants species, researchers used strong acid such as HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub> or HCl and strong acid such as KOH or NaOH to adjust the initial pH of the culture medium (McLay 1976; Akçin *et al.* 1994; Soltan and Rashed 2003; Awuah 2006). The initial pH of the effluent from the anaerobic pond used in this study was 6.8. To obtain the desired pH, the effluent from the anaerobic pond was spiked with sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) or sodium hydroxide (NaOH) depending on the pH level to be achieved.

The number of plants was counted in each pond at the beginning, the seventh, the fourteenth and the twenty-first day. Samples were taken at the same days for the measurement of total suspended solids (TSS), COD, BOD<sub>5</sub>, nitrate, nitrite, orthophosphates and Total Nitrogen Kjeldahl (TNK). The plant fresh weight (FW) and the faecal coliforms content of the medium were measured at the beginning and the end of the experiment. The environmental parameters such as pH, e<sub>H</sub>, conductivity, dissolved oxygen, and temperature and light intensity were measured on daily basis.

The significance of the relation between the pH, growth rate and removal performances of the water hyacinth was studied by means of statistical analysis using p-values by correlation matrix.

## 5.3 Results and discussion

### 5.3.1. Performance of the anaerobic treatment

The characteristics of the raw wastewater and anaerobic pond effluent are shown in table 3.1. The characteristics of the raw wastewater were in the limits of a medium strength domestic wastewater as classified by Metcalf and Eddy (Metcalf and Eddy 2003). The average BOD and COD in the raw water were respectively 516.9 mg/l and 218 mg/l (Table 5.1).

**Table 5. 1 : Raw wastewater and anaerobic pond effluent**

Parameters	Unit	Raw sewage	Effluent from the actual anaerobic pond	Removal rate %
Temperature	°C	26.8 ± 0.1	28.3 ± 0.1	
pH		6.469 ± 0.01	6.773 ± 0.001	
e <sub>H</sub>		24.1 ± 0.8	6.5 ± 0.1	
r <sub>H</sub>		13.75 ± 0.05	13.16 ± 0.03	
χ	μS/cm	745 ± 5	108 ± 2	
Turbidity	NTU	150.7 ± 1.5	72.5 ± 1.3	52%
COD	mg /l	516.9 ± 25	175.4 ± 7	66%
BOD <sub>5</sub>	mg /l	218 ± 35	101 ± 2	54%
MES	mg/l	160 ± 2	75 ± 0.5	53%
NTK	mg/l	20.86 ± 0.5	15.7 ± 0.3	25%
N-NO <sub>3</sub> <sup>-</sup>	mg/l	1.56 ± 0.01	1.1 ± 0.0	29%
N-NO <sub>2</sub> <sup>-</sup>	mg/l	0.000 ± 0.000	0.22 ± 0.00	
P-PO <sub>4</sub> <sup>3-</sup>	mg/l	26.6 ± 0.500	5.55 ± 0.10	79%
Faecal Coliforms	/100ml	1.05E+05 ± 465	1.57E+04 ± 165	85%

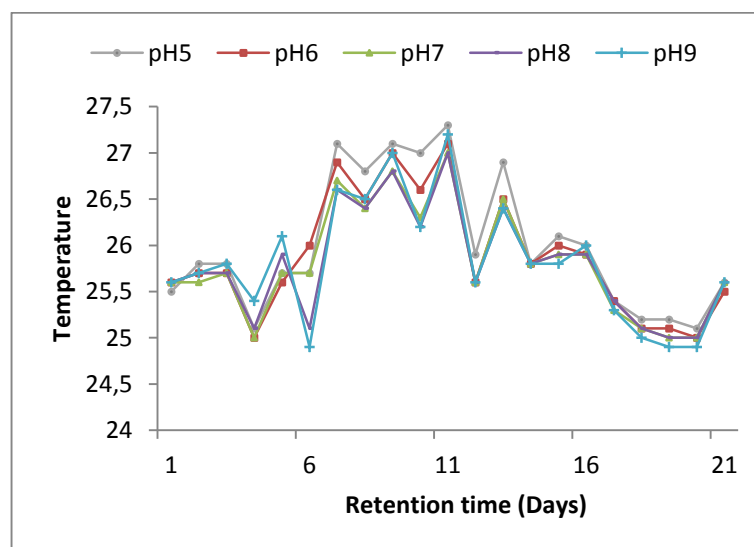
In the anaerobic pond, removals of 66% of COD, 56% of BOD<sub>5</sub> and 53% of TSS removals were achieved. These removal rates were lower than in our previous experiment (Chapter 4).

With the same experimental set up and treatment conditions, the organics loads and suspended solids removed from the medium strength wastewater were lower than that achieved by the anaerobic ponds with the high strength wastewater. This result confirmed that, in anaerobic ponds, the removal rate increased with an increase in the organic loading rate. The quality of the effluent from the anaerobic pond had an average BOD of 101 mg/l and COD of 175.4 mg/l.

### 5.3.2. pH and evolution of the environment parameters

#### Temperature

The temperature conditions in the different water hyacinth ponds are presented in Figure 5.1. The water temperature varied between 24.9 °C and 27.3°C in all the ponds with an average temperature of  $25.9 \pm 0.6$  throughout the experimental period.

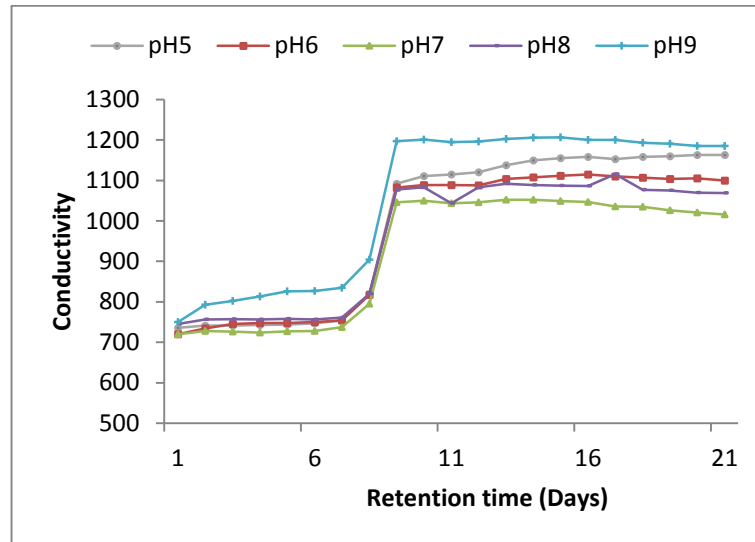


**Figure 5. 1 : Evolution of temperature in ponds**

The water temperature showed reasonably low variation and stayed within the optimum temperature range (22°C to 30°C) for water hyacinth growth (Allgayer 2006). The pattern of temperature changes was almost the same in all ponds. The changes in temperature did not depend on the initial pH of the pond but were related to the weather conditions.

**Electrical conductivity**

According to Figure 5.2, an increase in the electrical conductivity was observed in all ponds from an average value of  $734 \pm 11 \mu\text{S}/\text{cm}$  at the beginning to  $1007 \pm 62 \mu\text{S}/\text{cm}$  at the end of the experiment.



**Figure 5. 2 : Evolution of electrical conductivity in ponds**

The evolution of the electrical conductivity followed the same trend of steadily increasing in all the ponds, with a sudden increase in electrical conductivity in all basins between the seventh and the ninth day. It can be observed from Figure 5.2 that the lowest electrical conductivity was always observed in pond pH7, which has received the lowest quantity of NaOH to spike the wastewater. It was followed by the pond pH 8, pond pH 6, pond pH 5 and pond pH 9. The values of electrical conductivities in ponds seemed to be related to the quantity of sulphuric acid ( $\text{H}_2\text{SO}_4$ ) or sodium hydroxide (NaOH) used to spike the influent water.

**Turbidity**

As indicated in the Figure 5.3, the initial turbidity of  $72.5 \pm 1.3 \text{ NTU}$  dropped progressively from the first day to the eighth day where it reached average values of  $2.5 \pm 1.3 \text{ NTU}$ . This was due to the rapid settling of suspended solids. From the eighth day, the turbidity value passed from an average of  $3.81 \text{ NTU}$  to

23.36 NTU in the ponds with pH 9, and remained around this value till the end of the experiment. This was due mainly to the growth of algae biomass in ponds with pH 9, which remained in ponds till the end of experiment due to the low growth rate of water hyacinth observed.

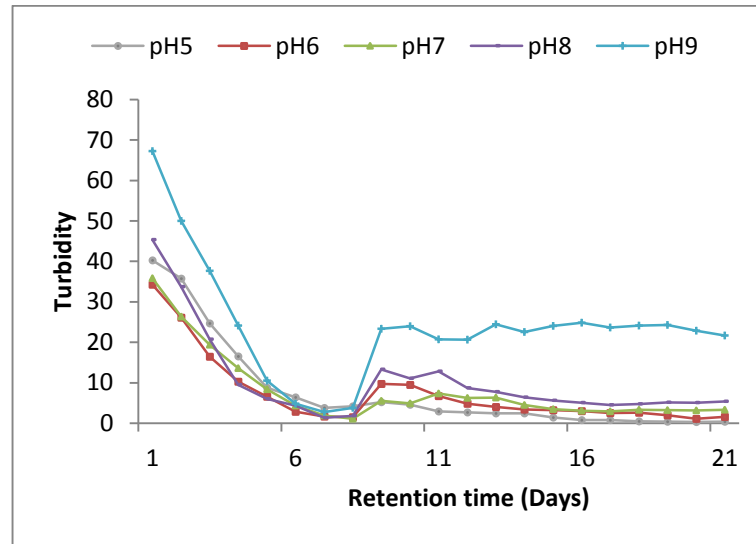


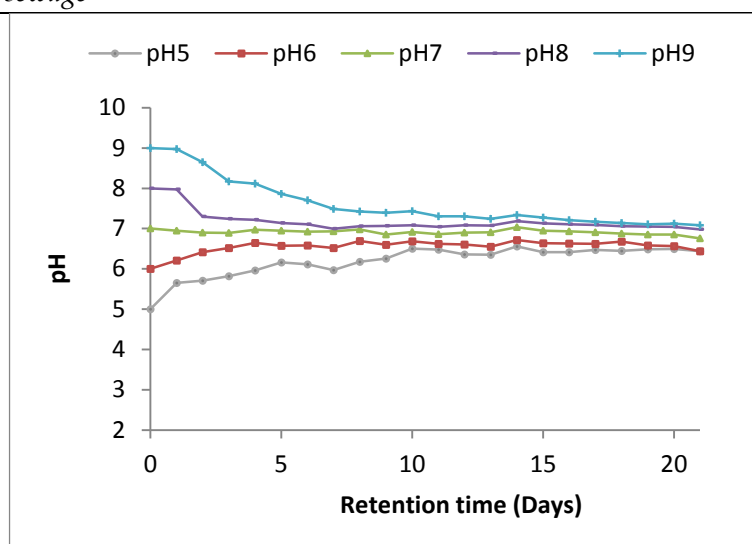
Figure 5. 3 : Evolution of electrical conductivity in ponds

### 5.3.3. pH and evolution of pH in ponds

The evolution of pH in ponds was highly related to the influent pH (Figure 5.4). In ponds with acidic initial pH, the daily recorded pH values increased rapidly the first days of the experiment. From the fifth day, the values continued increasing, but slowly, to reach an average pH of 6.44 in both ponds with initial pH of 5 and 6.

In contrast, in ponds with pH 8 and pH 9, the pH dropped, following almost the reverse trend compared to that of acidic influent water. At the end of the 21 days retention time, the average pH values recorded were 6.98 and 7.08 respectively in ponds with initial pH 8 and pH 9.

In ponds with pH 7, a decrease in pH values was observed but it was not pronounced. The pH passed from 7 to an average value of 6.75 at the end of the experiment.

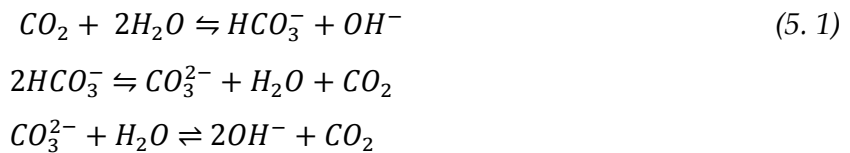


**Figure 5. 4 : Evolution of pH in ponds**

It has been observed that all the pH values converged toward pH values in the range of 6.4 and 7.1. Water hyacinth seemed to find this range of pH values optimal for their growth. This range is closer to optimum range for water hyacinth growth observed in previous studies. In fact Balasooriya et al. (1984) has reported, by studying water hyacinth growing in different water streams polluted by certain industrial effluents water, that optimum hyacinth growth occurred at pH within 6.0 and 7.0. Delgado et al. (1994), meanwhile, found this optimum growth occurring between pH ranges of 6.7 to 7.3 with an experiment carried out in a greenhouse at temperature between 28°C and 30°C using slurry containing pig manure as the nutrient source.

It has been observed here that, when the initial pH is not within the optimal range for the plants growth, but within the levels of pH 4 to pH 10 which they can tolerate for their survival as stated by Center *et al.*(2002), water hyacinth seems to have the ability to adjust the medium pH to their requirement. This adjustment can be associated to the changes in carbon-equilibrium states (Kim and Kim 2000); the observation of Kim and Kim was limited to water hyacinth ponds receiving alkaline effluent from algae ponds, the observation here confirmed that the adjustment held for basic as well as acidic influents.

It is known, the carbonate ions ( $\text{CO}_3^{2-}$ ) and the bicarbonate ions ( $\text{HCO}_3^-$ ) act as the primary buffer for most natural waters. Reactions that produce or consume carbon dioxide ( $\text{CO}_2$ ) may alter the pH temporarily until equilibrium with the atmospheric  $\text{CO}_2$  is re-established (Gilmour, 1992). The drop in pH under alkaline conditions could then be due to the inability of water hyacinth to use up all the  $\text{CO}_2$  produced during respiration. Then, the  $\text{CO}_2$  passes into the culturing medium through plant roots. On the other hand, under acidic conditions, water hyacinth consumed the  $\text{CO}_2$  at higher rate than it was produced by respiration. This will result in the dissociation of carbonate and bicarbonate ions by the reaction in Eq.5.1:



Water hyacinth will fix the molecules of  $\text{CO}_2$  formed, whilst the hydroxide ions ( $\text{OH}^-$ ) produced are used to increase the pH as alkaline conditions are created in algae ponds (Pearson *et al.* 1987; Henze *et al.* 2001).

#### 5.3.4. pH and organic matter and nutrient removal

According to Figure 5.5 (a) and (b) and the analysis of Table 5.2, COD and  $\text{BOD}_5$  removal in water hyacinth ponds were highly related to influent pH ( $p < 0.02$ ) even though the cumulative removal rate followed almost the same trend in all ponds. The changes, with regard to initial water pH, showed that the removal of carbon pollution from alkaline water became more and more difficult for water hyacinth with an increase in influent water alkalinity. An increase in the influent pH led to a decrease in carbon pollution removal performance. The same trend was observed when influent pH was becoming more acidic. However, water hyacinth had better performances in carbon pollution removal in acidic water than alkaline water.

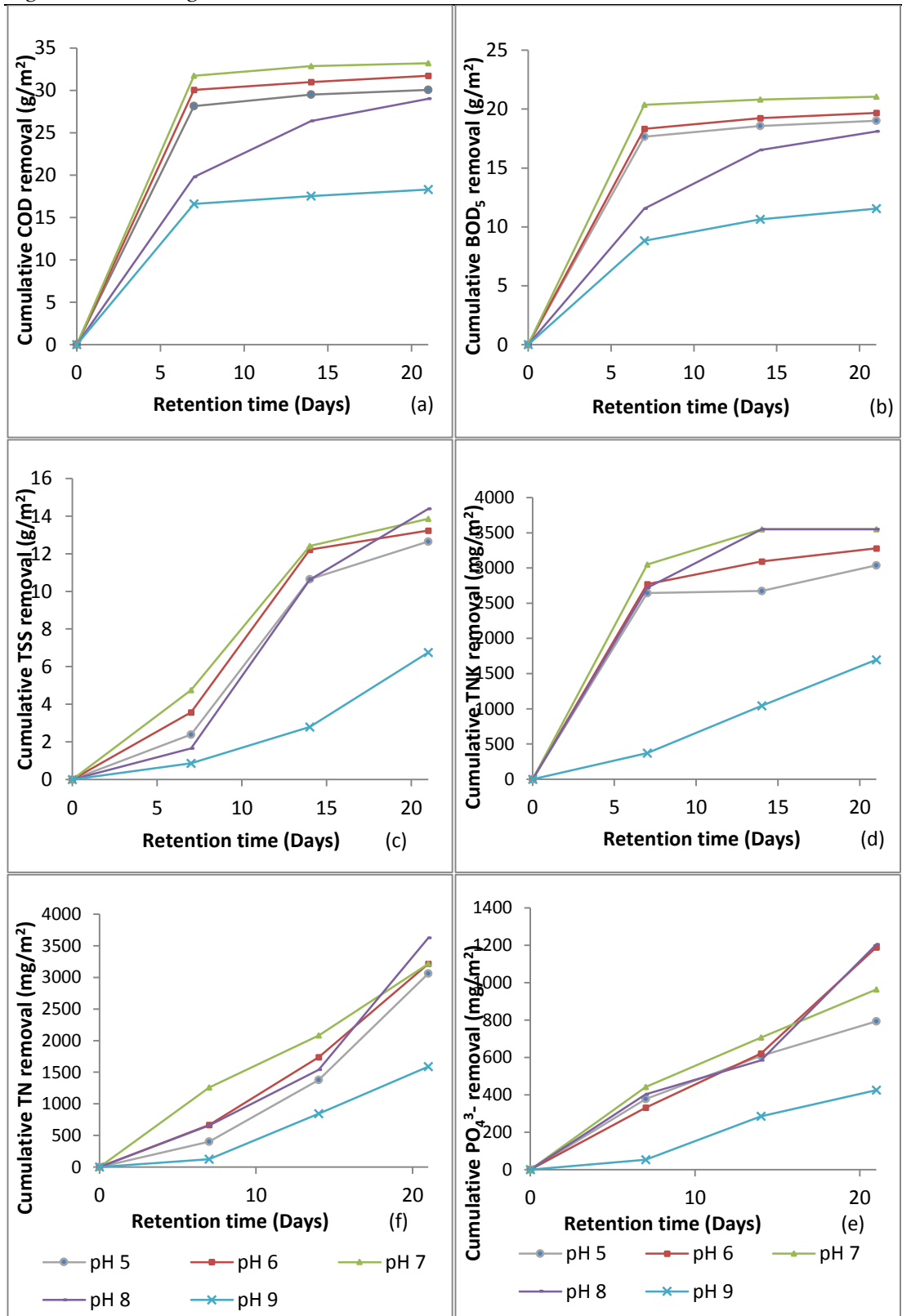


Figure 5.5 : Performance changes of water hyacinth with influent at different pH

In fact, with an influent pH 5, the overall removal of COD was 30.1 g/m<sup>2</sup> while 18.3 g/m<sup>2</sup> was registered with a pH 9. The influent water with a neutral pH showed the best removal performance.

From the analysis of Table 5.2, the carbon pollution removal was not significantly related to the retention time ( $p > 0.3$ ). This is confirmed by Figure 5.5 (a) and (b). By looking at Figure 5.5 (a) and (b), with regard to the retention time, it can be observed that the major part of the organic matter was removed within the first seven days. Indeed, the average influent COD of 175.4 mg/l was reduced after seven days retention, to values ranging from 35 mg/l to 102 mg/l at a removal rates of 17 g/m<sup>2</sup> to 32 g/m<sup>2</sup>.

**Table 5. 2 : p-values of removal performances in relation to pH**

Variables	pH	RT	COD	BOD <sub>5</sub>	TNK	TN	PO43-
pH	<b>0</b>	1.000	<b>0.002</b>	<b>0.003</b>	0.058	0.287	0.500
RT	1.000	<b>0</b>	0.413	0.342	0.253	<b>0.001</b>	<b>&lt; 0.0001</b>
COD	<b>0.002</b>	0.413	<b>0</b>	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>0.010</b>	<b>0.029</b>
BOD <sub>5</sub>	<b>0.003</b>	0.342	<b>&lt; 0.0001</b>	<b>0</b>	<b>&lt; 0.0001</b>	<b>0.009</b>	<b>0.023</b>
TNK	0.058	0.253	<b>&lt; 0.0001</b>	<b>&lt; 0.0001</b>	<b>0</b>	<b>0.002</b>	<b>0.012</b>
TN	0.287	<b>0.001</b>	<b>0.010</b>	<b>0.009</b>	<b>0.002</b>	<b>0</b>	<b>&lt; 0.0001</b>
PO43-	0.500	<b>&lt; 0.0001</b>	<b>0.029</b>	<b>0.023</b>	<b>0.012</b>	<b>&lt; 0.0001</b>	<b>0</b>

*Values in bold are different from 0 with a significance level  $\alpha=0.05$*

The highest COD removal within these seven days was achieved in ponds with pH 7 and this reduction represented 96% of the total COD removed during the experimental period. Thus, only an average of 6 mg/l of COD has been removed from the ponds with pH 7 from the seventh to the twenty-first day bringing down the COD to 29 mg/l at the end of the period of experiment. Similarly, the best removal of BOD was achieved with ponds with pH 7 and the major part has been removed within the first seven days. An average BOD of 20.4 g/m<sup>2</sup> was removed in these ponds within seven days with a total removal of 21.0 g/m<sup>2</sup> at the end of the 21 days of retention. The lower removal of 18.3 g/m<sup>2</sup> was observed in ponds with pH 9 at the end of the experimental period.

The high removal observed here within the first seven days is consistent with results of the previous experiments conducted on the same subject (Chapter 4), that the major part of the organic loads are removed in water hyacinth ponds within seven days retention time; therefore there is no need for ponds to have longer retention, the optimal retention time for carbon pollution removal is seven days. Increasing retention time added very little to efficiency with regard to the organic load removal. It may be worthwhile to have two ponds with retention time of about seven days than to have a pond with higher retention time.

The total suspended solids (TSS) removal rate was optimum in ponds with influent pH 7, even though the overall removal of TSS at the end the experimental period was higher in ponds with pH 8 (Figure 5.5 (c)). After seven days retention, the highest reduction in TSS of 4.8 g/m<sup>2</sup> was observed in ponds with pH 7. This rate changed to 12.4 g/m<sup>2</sup> on the fourteenth day and then 13.9 g/m<sup>2</sup> at the end of the experiment. The highest reduction in TSS in ponds with pH 8 was observed between the fourteenth and the twenty-first day for those ponds.

Figure 5.5 (d) and (e) show the TNK and TN cumulative removal in ponds at the different pH as function of retention time. Apart from the ponds with influent pH 9, the major part of TNK was removed within seven days retention time. The maximum removal of 3050 mg/m<sup>2</sup> achieved within this period was at pH 7, which also had the highest overall removal of 3552 mg/m<sup>2</sup> at the end of the experiment. The lowest removal rate of 1697 mg/m<sup>2</sup> at the end of the experimental period was observed in ponds with influent pH 9. The removal of TNK seemed to not be significantly ( $p > 0.25$ ) related to the retention time (Table 5.2), even though the trend of the cumulative removal in ponds with pH 9 seems to be time dependent (Figure 5.5 (d)). TNK cumulative removal rate was used to measure the nitrification rate. The nitrification rate correlated with the carbon pollutant removal rate ( $p < 0.0001$ ) but the correlation with the influent pH seemed not very significant ( $p > 0.058$ ). The low effect of pH on nitrification rate

may be due to the rapid adjustment of the medium pH by water hyacinth to values close to optimum pH range for nitrification, 7 to 8 (Caicedo Bejarano 2005).

Analysis of the trends of the curves of Figure 5.5 (e) showed that the optimum TN cumulative removal was achieved in ponds with pH 7, even though the ponds with pH 8 showed the highest overall removal of 3623 mg/m<sup>2</sup> at the end of the period of the experiment. In fact, in pH 7 the removal rate was progressive with average values of 1256 mg/m<sup>2</sup>, 2083 mg/m<sup>2</sup> and 3214 mg/m<sup>2</sup> the 7<sup>th</sup>, 14<sup>th</sup>, and 21<sup>st</sup> day, respectively. In pH 8, a sudden increase of the TN removal rate from 1539 mg/m<sup>2</sup> the 14<sup>th</sup> day to 3623 mg/m<sup>2</sup> at the end of the experiment was observed. This change may be explained by the high plant growth observed in these ponds those last days after the adjustment of the pH in ponds. The TN removal rate was not correlated to the pH ( $p=0.287$ ) but it was significantly correlated to the retention time, the organic loads and the phosphate removal rates ( $p=0.001$ ). The maximum TN removed represented 94.1%, which was higher than 83.26% removal reported by (Maharjan and Ming 2012) after 4 weeks retention time of water hyacinth ponds receiving fresh wastewater in Nepal. This difference may be due to the high initial TN concentration (192.9 mg/l) of the raw wastewater used by these researchers.

Figure 3.2 (f) shows the orthophosphate removal rate in the different ponds as function of time. The orthophosphate showed almost the same trend with time as the TN removal for the different influent pH values. The optimum removal trend was observed at influent pH 7, while the highest overall cumulative orthophosphate of 1204 mg/m<sup>2</sup> was achieved with influent pH 8. The orthophosphate removal rate seemed not related to pH ( $p= 0.5$ ) but to the retention time ( $p = 0.001$ ), the TN removal ( $p < 0.0001$ ) rate and the organic load removal rate ( $p < 0.03$ ).

In general it was observed that organic and nutrients loads removal rates decreased when influent pH increased from pH 7 to alkaline pH or decreased from pH 7 to acidic pH.

### 5.3.5. pH and microbial pollution removal

From the analysis of Table 3.3, it appears from the coliform count of the effluents of the ponds pH 5, pH 6 and pH 8 that there was an increase in coliform number in the ponds, despite the long retention time and the presence of water hyacinth. In ponds with initial pH 7 and pH 9, 89% and 84% coliform removal was achieved.

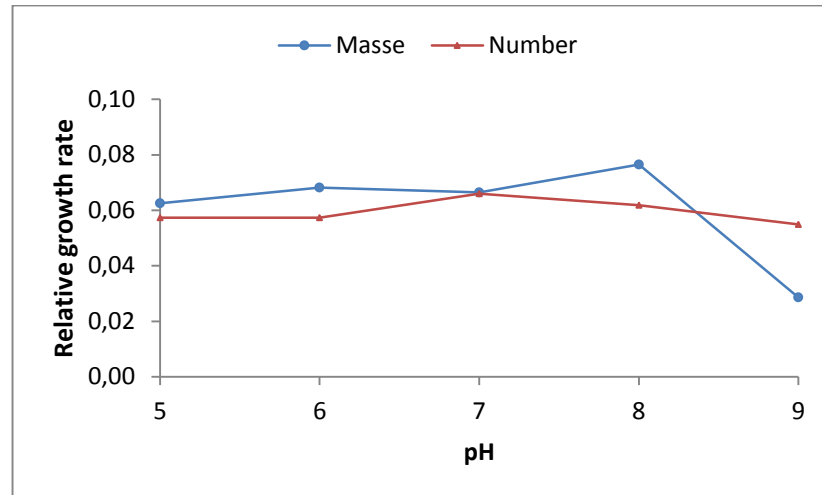
**Table 5. 3 : Coliform count in influent and effluent of ponds**

Pond	Effluent from anaerobic pond	Ponds pH 5	Ponds pH 6	Ponds pH 7	Ponds pH 8	Ponds pH 9
Coliforms Number	15700	46 000	19 600	1 800	20 000	2 500

It has been reported that coliforms can multiply in treatment facilities or watercourses (Gibbs *et al.* 1997) depending on the environmental conditions. The increase of the coliform number observed can be associated with their multiplication in ponds, which is related to the conditions in the water hyacinth ponds. In fact after the day 3, the observed pH in ponds fell within the range of the optimum pH for faecal bacterial growth which is from 6.5 to 7.5 (Awuah 2006). Also, the effects of high pH and sunlight which have been stated to be one of the most beneficial for disinfection in ponds (Gersberg and Silvaggio 1992; Awuah *et al.* 2004) cannot be expected from water hyacinth ponds, as in these ponds acidic conditions prevailed. Furthermore, some works suggest that the addition of nutrients like glucose and saline increase the survival chances of bacteria under both light and dark conditions (Liltved and Landfald 2000; Van der Steen *et al.* 2000). This may explain the survival of coliforms and their multiplication in this experiment. The removal observed in ponds with initial pH 7 is due to the very low quantity of solute added to the influent wastewater

for pH spiking. As for the ponds with initial pH 9, the high algae growth and the low water hyacinth growth in the ponds may have improved the DO content and the sunlight effect on ponds leading to coliform removal.

### 5.3.6. pH and plant biomass production



**Figure 5. 6 :** Plant relative growth related to the masse and number of plants as function of influent pH

The water hyacinth relative growth, as measured by biomass fresh weight (FW), varied between 0.029 and 0.076 with the highest value observed in ponds with pH 8; but the biggest number of plants was observed in ponds with initial pH 7. In fact the relative growth rate, as measured by the number of plants, varied between 0.055 and 0.066 with the optimum value observed in ponds with pH 7. The lowest growth rate was observed in ponds with pH 9.

The mean initial plant unit wet weight was  $42.5 \text{ g} \pm 2.6 \text{ g}$ . The final unit plant mass varied between 25.6 g and 56.2 g. The peak values of unit fresh weight were observed in ponds with initial pH 6 and 8. This can be seen by the higher values of relative growth, as measured by fresh biomass weight and the size of the observed plants in ponds.

## **5.4 Conclusions**

The effect of pH on water hyacinth ponds for domestic wastewater treatment was carried in pilot scale ponds under batch flow. The influent domestic wastewater, pretreated anaerobically, was spiked with H<sub>2</sub>SO<sub>4</sub> or NaOH to get the desired initial pH for water hyacinth ponds. Five different initial pHs (pH 5, pH 6, pH 7, pH 8 and pH 9) were tested. The anaerobic treatment was able to remove 66% of COD, 56% of BOD<sub>5</sub> and 53% of TSS after five days retention time.

The observed pH in water hyacinth ponds for wastewater treatment ranged between 6.4 and 7.1. When the initial pH values move outside this interval; the plants regulated the pH of the medium to within this range of 6.4 to 7.1 during the treatment processes. This adjustment affected the performances of the ponds.

The pollutant removal rate depended on the influent pH. An influent pH value around neutral value was optimum for treatment processes in water hyacinth ponds, meanwhile the ponds with influent pH 6 and pH 8 showed higher an overall total nitrogen and phosphate removal at the end of the experimental period, when the retention time was over fourteen days.

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An increase in the faecal coliforms content was observed in the ponds with influent pH 5, pH 6 and pH 8; a removal is achieved in ponds with initial pH 7 and pH 9.

# 6

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## **Heavy Metal Removal by water hyacinth: kinetic and performance**

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### **6.1 Introduction**

Heavy metals are a potential risk to public health and environment. Due to the fact that they do not decay, they are potentially persistent in environment and readily accumulated to toxic level (Chung *et al.* 2011; Dotro *et al.* 2012).

Several scientific studies have showed the effectiveness of aquatic floating macrophytes in removing pollutants, especially heavy metals from aqueous solution (Lesage *et al.* 2007; Visermanee *et al.* 2008). Most of these studies aimed to enhance the maximum uptake ability of these plants (Liu *et al.* 2007; Miao *et al.* 2007; Fibbi *et al.* 2011). There still however a lack of systematic studies regarding the kinetics of bioaccumulation at different metal concentrations and the effect of this accumulation on plant productivity and biomass reuse. Also most of the studies used low concentrations of metal, while it has been shown that the concentration of Zn, Fe and Cu in municipal wastewater can be over 20 mg/l. For example, an analysis carried out on domestic wastewater characteristics in Cotonou showed it may contain Fe, Cu and Zn up to 87 mg/l, 45 mg/l, 23 mg/l, respectively (Houngkpè *et al.* 2013).

By the analytical approach used in this study, the kinetics of copper, iron and zinc bioaccumulation by water hyacinth and the plant biomass production rate were investigated as function of exposure time at different initial concentrations. This will aid in developing a tool that could help to estimate the biomass quality

and quantity and to identify the limit of water pollution at which the cultured plants biomass can be reused as animal food or in agriculture.

## 6.2 Materials and methods overview

The experiment was carried out on the University Campus of Abomey-Calavi (Benin - West Africa).

In the first place, anaerobically pretreated sewage from university halls of residence was used to fill different plastic containers. The content of each plastic container was polluted with the metal by adding the appropriate volume of salt metal solution to reach the different ranges of desired metal concentrations. However, the coagulation of the colloids in the wastewater by the metal cations resulted in the sewage treatment instead of polluting it with free metal ions. To avoid this coagulation problem, a synthetic culture solution was prepared to allow for plant growth and free metallic ions in solution. The mini-ponds type MP-3 filled with 20l of Hillman culture with fifteen days retention time monitored under batch flow condition, as described in chapter 3, were then used in the present experiment.

The solutions in the mini-ponds were polluted with salts of heavy metals to reach the desired initial experimental metal concentrations.

Different salts of metals such as  $\text{CuSO}_4$ ,  $\text{Cu}(\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  have been used in literature to pollute water with free metal ions (Jayaweera *et al.* 2007; Razinger *et al.* 2007; Jayaweera *et al.* 2008; Materazzi *et al.* 2012; Sekomo *et al.* 2012). In the present study, three salts of heavy metals from the manufacture Aldrige were used: copper sulphate ( $\text{CuSO}_4$ ), iron II and ammonium sulphate [ $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ ] and zinc sulphate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ).

The concentrations were: 2 mg/l, 5 mg/l, 10 mg/l and 20 mg/l for each metal. These concentration ranges were chosen taking into account not only the values commonly found in the sludge from individual facilities and possible dilutions when mixed with sewage, but also for the reduction which will occur in the

anaerobic pond during pre-treatment. The three metals were studied separately at these concentrations and mixtures of the three metals at the same concentrations were checked in order to analyse their combined effect.

A control container was planned to be put in place, but due to presence of copper at 2.89 mg/l in the composition of the synthetic medium, the control container, with blank culture medium, and the container used to check the concentration of 2mg/l of copper were actually the same.

Four mature healthy water hyacinth plants of similar size and height were prepared and introduced directly in each mini-pond as described in Chapter 3 (Figure 6.1). The culture medium used was not replaced during the whole period of experimentation.



**Figure 6. 1 :** *Experimental ponds at the beginning of the experiment*

Samples were taken of the medium were taken at the beginning, the third, seventh, eleventh and fifteenth day to check the metal content. The environmental parameters (temperature, pH, conductivity,  $e_H$  and turbidity) were measured every day when sunrise. The number of plants and the total fresh weight of plants in each container were determined at the beginning and

the end of the experiments. The metal content of plants biomass was measured at the end of the experiment.

At the end of the experimental period, the total biomass of water hyacinth was divided into two portions. The first portion was used to continue the experiment. The second portion was used to determine the metal accumulated in plants.

The initial metals content of the plants was not measured before the experiments started. Due the fact that the plants used were taken from the same medium, it was assumed, in the present study, that the initial concentrations of metals in plants were the same.

The relation between the plant relative growth, the initial concentration of metal in solution, the removal efficiency and plant biomass metal content was carried out using the statistical method of the principal component analysis (PCA) of the correlation matrix.

## **6.3 Results and discussions**

### **6.3.1. Environmental parameters**

The water temperature varied between 23.5°C and 25.0°C and the pH varied between 3.27 and 5.47 throughout the experimental period, with an average of 24.3°C and 4.4 respectively for temperature and pH.

The temperature showed a relatively low variation between ponds with observed values within the optimum range for growth of water hyacinth (Allgayer 2006) Mama 2010).

In most of the containers, the initial value of the pH (4.4) was the smallest value; an increase of pH was observed in all the ponds throughout the experimental period. This initial acidic condition was associated to the addition of metal salt to the medium, leading to a reduction of pH. During the experimental period, an increase of the pH was observed in ponds; this is in accordance with the

adjustment of the pH by water hyacinth to its optimum range, stated in the previous conclusion of chapter 5.

### 6.3.2. Kinetics of metal removal from solution

Two mathematical equations were used in this study to model the overall removal mechanism: the first order and second order models. In the first order equation, it is assumed that the metal removal rate is proportional to metal concentration  $C$  in the medium. It is given by:

$$\frac{dC}{dt} = -k_1 C \quad (6.1)$$

Where  $k_1$  is the rate constant (in day<sup>-1</sup>)

After integration of this equation we have:

$$\ln \frac{C}{C_0} = -k_1 t \quad (6.2)$$

In the second order equation it is assumed here that the rate of removal is proportional to the square of metal concentration in the medium

$$\frac{dC}{dt} = -k_2 C^2 \quad (6.3)$$

$k_2$  is the second order rate constant in l/mg.day. After integration the equation becomes:

$$\frac{1}{C} - \frac{1}{C_0} = k_2 t \quad (6.4)$$

The value of  $k_1$ ,  $k_2$  and  $R^2$  are determined and represented in Table 6.1 for Cu, Fe and Zn.

From Table 6.1, it appears from the comparison of the regression coefficient  $R^2$  that the first order kinetics fit better for the bioremediation of Zn and Fe removal when each of these metals was alone in water as well as in the mixture; for the Cu removal, the difference between the first order and the second order kinetic model regression coefficients  $R^2$  was found to be negligible.

**Table 6. 1 : Kinetic parameters for Cu, Fe and Zn removal by water hyacinth**

	First Order		Second order			First Order		Second order	
	k <sub>1</sub>	R <sup>2</sup>	k <sub>2</sub>	R <sup>2</sup>		k <sub>1</sub>	R <sup>2</sup>	k <sub>2</sub>	R <sup>2</sup>
<b>Cu2</b>	0.064	0.990	0.053	0.992	<b>Cu-M2</b>	0.048	0.983	0.042	0.973
<b>Cu5</b>	0.065	0.979	0.027	0.992	<b>Cu-M5</b>	0.050	0.966	0.019	0.991
<b>Cu10</b>	0.071	0.971	0.012	0.960	<b>Cu-M10</b>	0.057	0.978	0.008	0.994
<b>Cu20</b>	0.076	0.954	0.005	0.966	<b>Cu-M20</b>	0.067	0.961	0.004	0.991
<b>Fe2</b>	0.181	0.930	0.415	0.840	<b>Fe-M2</b>	0.227	0.907	0.806	0.749
<b>Fe5</b>	0.206	0.839	0.260	0.575	<b>Fe-M5</b>	0.250	0.910	0.487	0.726
<b>Fe10</b>	0.251	0.880	0.228	0.693	<b>Fe-M10</b>	0.289	0.925	0.409	0.769
<b>Fe20</b>	0.174	0.899	0.040	0.773	<b>Fe-M20</b>	0.221	0.867	0.084	0.606
<b>Zn2</b>	0.123	0.867	0.176	0.742	<b>Zn-M2</b>	0.155	0.883	0.294	0.734
<b>Zn5</b>	0.151	0.910	0.111	0.821	<b>Zn-M5</b>	0.183	0.888	0.180	0.717
<b>Zn10</b>	0.130	0.864	0.038	0.719	<b>Zn-M10</b>	0.162	0.869	0.066	0.690
<b>Zn20</b>	0.112	0.879	0.014	0.777	<b>Zn-M20</b>	0.153	0.859	0.028	0.676

From this table, it can be observed that the first order kinetic constant varied from 0.064/day to 0.076/day, 0.181/day to 0.251/day, 0.112/day to 0.151/day (Table 6.1) with a maximum removal percentage of 71%, 87% and 96% respectively for Cu, Zn and Fe when the metals were tested separately (Figure 6.2). In the mixtures, these constants ranged from 0.048/day to 0.067/day, 0.155/day to 0.183/day and from 0.221/day to 0.289/day with the maximum removal of 66%, 91% and 98% respectively for Cu, Zn and Fe.

In all the cases the kinetic constant of Fe was greater than that of Zn, which was in the range of twice that of the Cu. The removal of Fe is therefore faster than that of Zn followed by Cu. We can conclude that the preferential sequence of accumulation of toxic elements in water hyacinth was Fe>Zn>Cu.

This preferential accumulation rate of the different metals is in confirmation of the results obtained by Mishra, V. K. and B. Tripathi (2008) in their study.

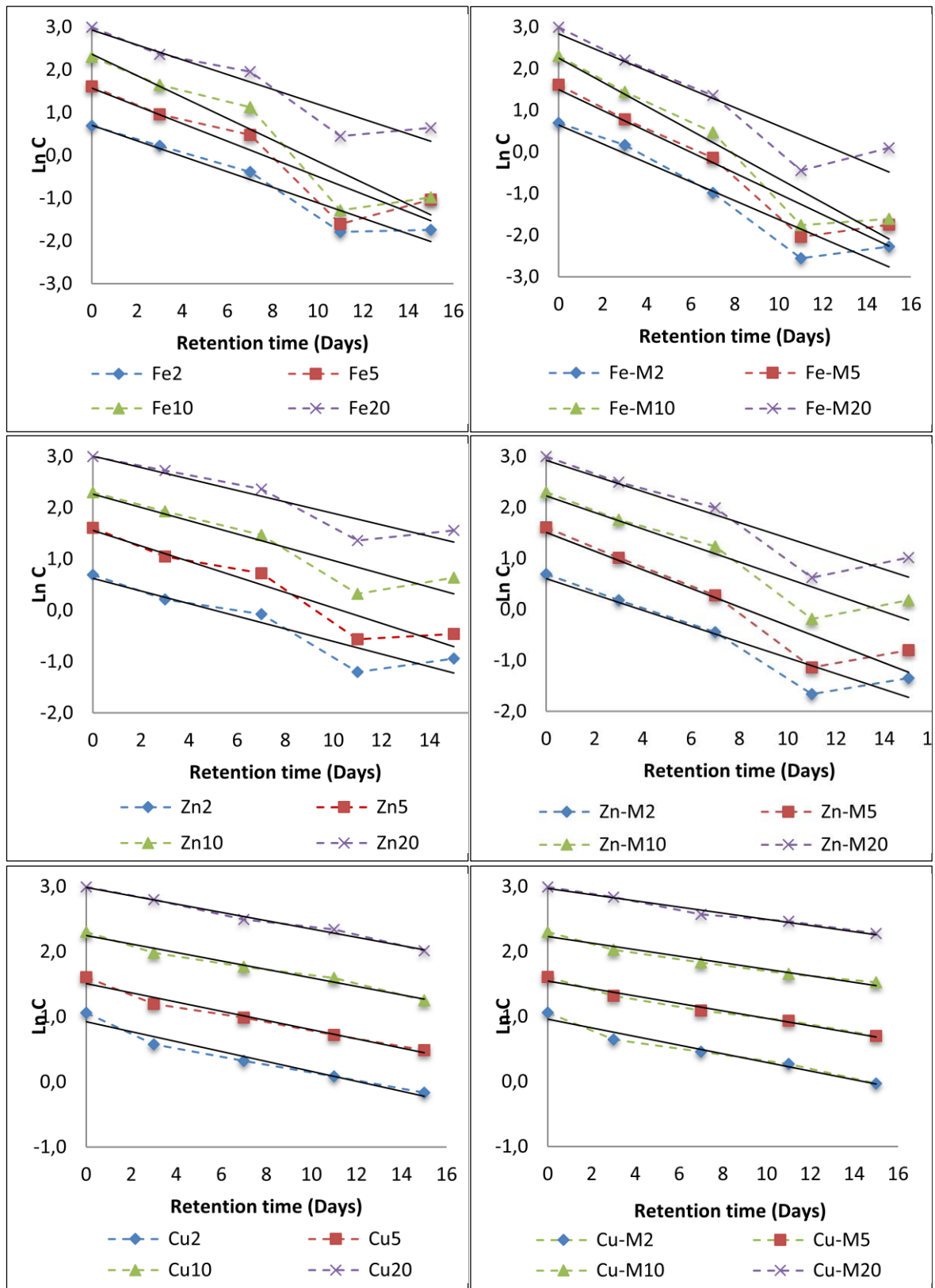


Figure 6. 2 : Metal removal kinetics models for Cu, Fe and Zn removal by water hyacinth

In fact, they demonstrated a preferential up-take of metals by macrophytes after testing the removal of five metals (Fe, Zn, Cu, Cr and Cd) from aqueous solution by *Pistia stratiotes* L. (water lettuce) and *Spirodela polyrrhiza* W.Koch (duckweed). Also, a similar observation was made by Ahmet and Erdal with *Lemna gibba* exposed to secondary effluent. The uptake of Fe was greater and faster during the first three days; this metal is then easily and readily adsorbed by water hyacinth.

Figure 6.2 shows the first order kinetics for the removal of the three metals from water. The metal removal kinetics as defined by the slopes of the straight lines fitted, are similar for the various different concentrations tested.

These curves revealed that the removal of Cu decreased with time up to the end of the experiment regardless the initial concentration. The residual concentrations of Fe and Zn in water decreased with time and reached a minimal value at the eleventh day; from the eleventh day, an increase of the residual concentration of the two metals have been observed. This can perhaps be explained by the fact that water hyacinth has reached the saturation levels for Fe and Zn at the 11<sup>th</sup> day and release of metals in water may have occurred from this point.

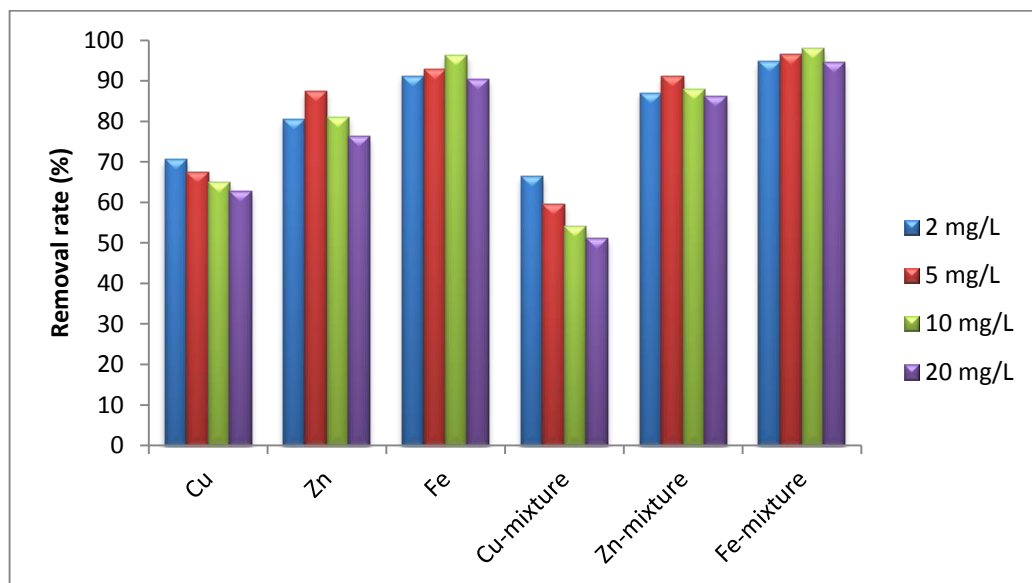
These confirmed the results obtained by Mishra, V. K. and B. Tripathi (2008) who observed an optimal removal at the twelfth day and a release at the fifteenth day of experiment.

These curves revealed that the removal of Cu decreased with time up to the end of the experiment regardless the initial concentration. The residual

The overall behaviour of the three metals did not change when they were mixed together in the aqueous solution; although the removal rate of Cu was slightly inhibited by the presence of Fe and Zn while the presence of the other toxic elements enhanced the removal rate of Zn and Fe.

### 6.3.3. Performance of metal removal from solution

The removal percentage of Cu decreased from 71% to 63% with an increase in the initial concentration of metals in water from 2mg/l to 20mg/l, which meant that the higher the initial concentration of Cu in water the lower the removal percentage (Figure 6.2 and Figure 6.3). This may be due to huge mass of metal that plants had to accumulate with high initial concentration of metal which made more difficult the removal by water hyacinth ponds. The Cu was the most toxic to the plants among the three metals, as a complete degeneration of the plants was observed at an initial concentration of 20mg/l. In spite of this degeneration, removal of the metal was observed. This suggested that the plant material can be used as bio-adsorbent for Cu removal.



**Figure 6. 3 : Percentage of metals removal from water**

An increase in the percentage of removal of Fe by water hyacinth from 91% at an initial concentration of 2mg/l to 96% at 10mg/l followed a decrease from this percentage to 90% at an initial concentration of 20mg/l was observed. The same phenomenon was observed with Zn, but the optimum removal percentage of 87% was observed at the initial concentration of 5mg/l (Figure 6.2 and Figure 6.3). The Fe was, probably, among the three metals the most easily assimilated by the water hyacinth.

In the solution containing the mixture of the three metals, an increase of the removal percentage of Fe and Zn was observed compared to the solution in which the metals were alone at the same initial concentration. The presence of the metals together seemed to enhance their accumulation by the plants.

The lowest removal percentage for the three metals, whether alone (63%, 76% and 90% respectively for Cu, Zn and Fe) or in mixture (51%, 86% and 94% respectively for Cu, Zn and Fe), was observed for an initial concentration of 20 mg/l; this initial concentration seemed to be more toxic for the plants (Figure 6.3) among the concentrations tested.

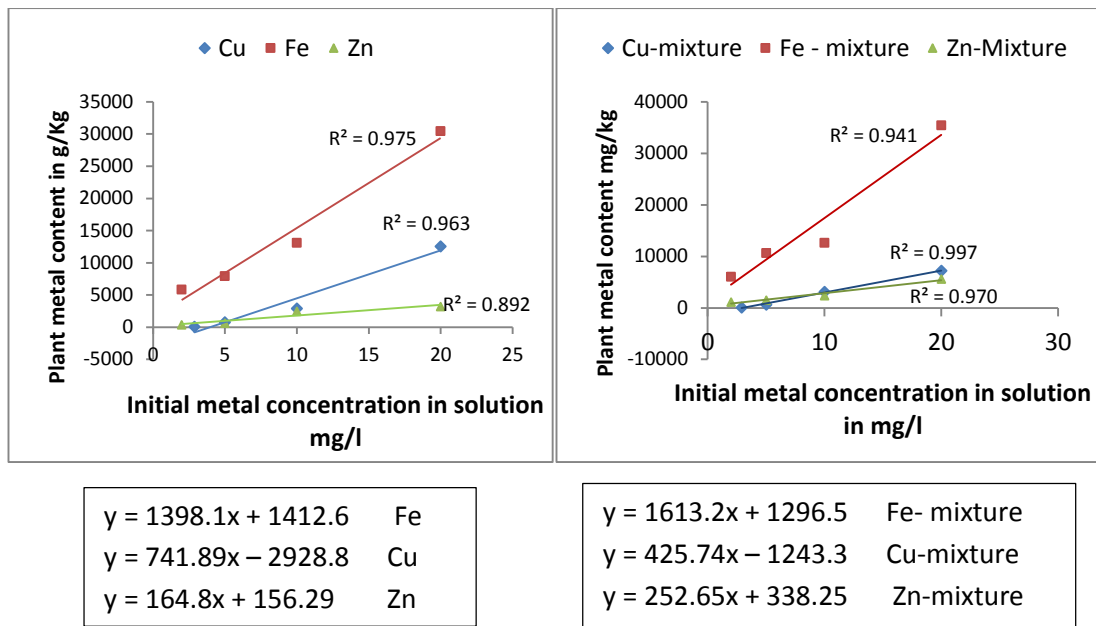
The high removal rates observed were in accordance with the findings of some researchers who had demonstrated in their study that water hyacinth was a hyper-accumulator of these metal pollutants (Jayaweera *et al.* 2007; Mishra and Tripathi 2008; Gakwavu *et al.* 2012).

#### **6.3.4. Metal accumulation in plants**

Figure 6.4 shows that there is a linearly increasing relationship between the metal accumulated in plants, expressed as function of dried weight (DW), and the quantity of metal removed from water, regardless the type of metal and whether it is alone or mixed with others in solution.

The observed pattern agreed with previous results obtained by Hassan, *et al.* while checking the uptake of Zn and Cd by water hyacinth separately and in a mixture solution (Hasan *et al.* 2007).

The linearity was expressed at high trend ( $R^2$  greater than 0.89) in all cases. The accumulated metal in plants biomass was significantly and linearly related to the initial metal content of the solution. This confirmed that, as previously said by some authors (Sheoran and Sheoran 2006), accumulation in plant biomass was the most important metal removal process in macrophyte ponds.



**Figure 6. 4:** Metal concentration in biomass as function of initial concentration of metal in solution

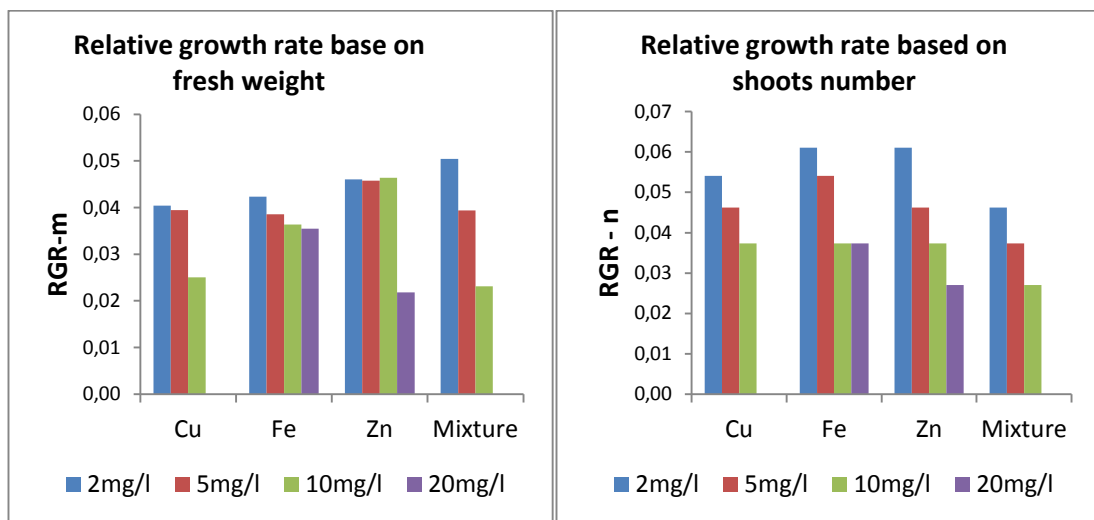
The Cu concentration in plants tissue increased from 120 mg/Kg DW for an initial concentration of 2.89 mg/l to 1256 mg/Kg DW for an initial concentration of 20 mg/l. The accumulated Zn and Fe by the water hyacinth increased respectively from 390 mg/Kg to 3212 mg/Kg DW and from 5876 mg/Kg DW to 30464 mg/Kg DW with initial concentrations of solution varying from 2 mg/l to 20 mg/l when the metals are checked alone. In the mixture, the metal concentrations in plant tissues varied from 110.22 to 7238.19 mg/Kg DW, 1151.66 to 5610.44 mg/Kg DW and 6082.89 to 35464.62 mg/Kg W for Cu, Zn and Fe respectively for the same initial concentrations. Water hyacinth was able to accumulate the three metals simultaneously, at even a higher rate, for Fe and Zn, than when the metals were in separate solution.

From the study of Hassan, et al (2007), it appeared that water hyacinth was able to achieve accumulation in their lower parts of 1291.0 mg/kg DW to 10 300.0 mg/kg DW for initial concentrations varying from 2.0 mg/l to 12.0 mg/l after sixteen days of experiment. The observed accumulations were higher than those obtained in the present experiment. This may be explained by the fact that the lower part considered by their study is limited to the root, and the population of

water hyacinth was limited to one plant and no new shoots of water hyacinth were observed.

### 6.3.5. Plant growth

The water hyacinth growth rate was studied based on number of clones and on the wet weight. The results are shown in Figure 6.5. In general, the relative growth rate of the plants increased with the initial concentration of the metal pollutants in solution regardless the type of the metal.



**Figure 6. 5 :** Relative growth rate of water hyacinth as function of type of metal and its concentration in solution

The highest relative growth rate of 0.050/day was observed with the mixture solution at initial concentration of 2mg/l of three metals; the lowest positive growth based on mass rate of 0.022/day was achieved when the culture medium contained 20 mg/l of Zn. These represented a biomass doubling time varying from 13 days to 32 days. However, the growth rates were not determined for the solutions containing 20 mg/l of copper because the complete degeneration of the plants meant a negative growth rate (Figure 6.5). The unit plants masses varied from 40.9 g/plant to 70.4 g/plant.

In solutions with the same concentration of the different metals, the relative growth rate based on the numbers of plants varied between 0.027/day to

0.061/day which represented a doubling in shoot numbers from 11 days to 26 days.

This result is in disagreement with Mishra, V. K. and B. Tripathi (2008) who stated that macrophytes accumulate heavy metals (Fe, Cu, Zn) in their body without the production of any toxicity or reduction in growth. This conclusion may be due to the fact that Mishra, V. K. and B. Tripathi (2008) did not measure the growth rate of the plants, but only their Chlorophyll, protein and sugar content.

The reduction in growth observed may be attributed to the production of physiological and biochemical responses due to the accumulation of heavy metal, as stated by several researchers (Satyakala and Jamil, 1992; Chandra and Kulshreshtha, 2004; Shankers et al., 2005).

Another observation was that, despite the low growth rate based on the number of shoots for the solution containing the mixture of the three metals, good growth rate based on the mass was recorded. This result confirmed the conclusion of Koné (2002) saying that macrophytes prioritize increasing their number of shoots in the appropriate development environments, and the growth of the existing biomass in harsh environments. In fact in favorable development conditions, macrophytes multiply easily themselves to cover the available surface due to the availability of all their development requirements. In contrary, in difficult development conditions, macrophytes fight for their survival and maintenance and by doing that increase their size (height and weight).

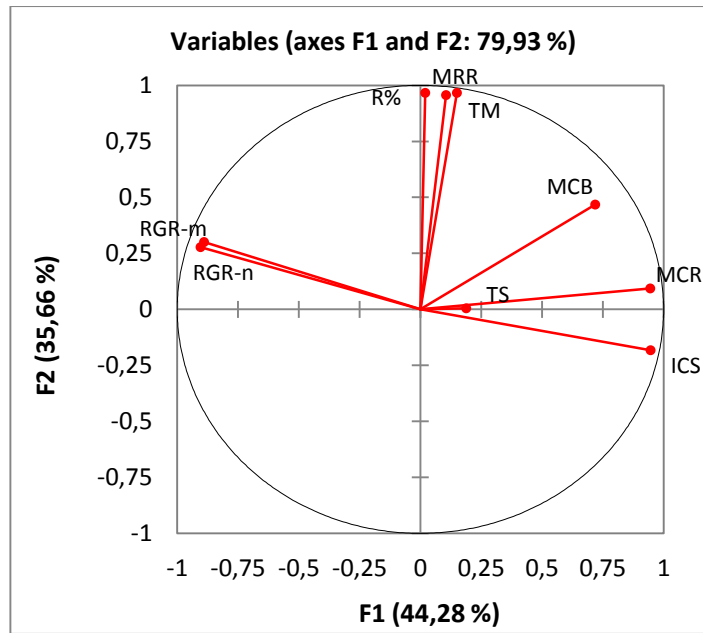
Also, this drop in growth rate observed will affect biomass production during treatment and by then reduce the plant biomass which will be available for other pollutants removal.

### 6.3.6. Analysis of the relation between plant growth, metal removal and accumulation in biomass

The first two principal components explained more 79% of the total variance in the data (Figure 6.6). The analysis of the data in this plan is then acceptable. The two axes showed a good representation and distribution of the variables studied. The first axis (F1), which explains 44.3 % of the total variance, revealed an association of the removal rates and the type of metal. The second axis (F2), explaining 35.7% of the total variance, defined at its positive pole the quantity of metal removed, the metal concentration in the biomass of plants and the initial concentration and at its negative pole the relative growth rates.

**Table 6. 2 : Correlation matrices between variables and factors**

Variables	ICS	TM	TS	R%	MCB	RGR-m	MRR	RGR-n	MCR
ICS	<b>1</b>	-0.013	0.000	-0.156	<b>0.582</b>	<b>-0.852</b>	-0.113	<b>-0.855</b>	<b>0.944</b>
TM	-0.013	<b>1</b>	0.000	<b>0.920</b>	<b>0.545</b>	0.147	<b>0.921</b>	0.147	0.231
TS	0.000	0.000	<b>1</b>	0.016	0.029	-0.198	0.154	-0.384	0.003
R%	-0.156	<b>0.920</b>	0.016	<b>1</b>	0.375	0.252	<b>0.933</b>	0.222	0.130
MCB	<b>0.582</b>	<b>0.545</b>	0.029	0.375	<b>1</b>	<b>-0.432</b>	<b>0.456</b>	<b>-0.434</b>	<b>0.713</b>
RGR-m	<b>-0.852</b>	0.147	-0.198	0.252	<b>-0.432</b>	<b>1</b>	0.144	<b>0.921</b>	<b>-0.752</b>
MRR	-0.113	<b>0.921</b>	0.154	<b>0.933</b>	<b>0.456</b>	0.144	<b>1</b>	0.107	0.140
RGR-n	<b>-0.855</b>	0.147	-0.384	0.222	<b>-0.434</b>	<b>0.921</b>	0.107	<b>1</b>	<b>-0.765</b>
MCR	<b>0.944</b>	0.231	0.003	0.130	<b>0.713</b>	<b>-0.752</b>	0.140	<b>-0.765</b>	<b>1</b>
<i>Values in bold are different from 0 with a significance level alpha=0,05</i>									
ICS	Initial concentration in solution								
TM	Type of Metal (Cu, Zn or Fe)								
TS	Type of solution (mixture of 3 metal or 1 metal in solution)								
R%	Percentage of removal								
MCB	Metal Content of the biomass (mg/kg)								
RGR-m	Plant relative growth rate with masse (Ln(mf/mi)/t)								
MRR	Average Metal Removal rate (ln(Co/C)/t)								
RGR-n	Plant relative growth rate based on shoots numbers (Ln(mf/mi)/t)								
MCR	Metal concentration removed								



**Figure 6. 6** : Projection of the variables into the factorial plan (F1, F2)

From the analysis of the Figure 6.6, it appeared that the plant growth rates were highly affected by the quantity of the metal accumulated in the biomass of the plants with a significance level of 0.05. This quantity depended on the concentration of the metal removed from solution (0.713) which was, in turn, correlated to the initial metal concentration in water (0.944), all at a significance greater than 0.05. Hence, the result showed that the plant growth rates were significantly affected by the initial concentration of metal in solution with a significance level of 0.05 (Table 6.2). The significant correlation between the metal removed from solution and the metal accumulated in the biomass of the plants revealed that the major part of the metal removed was in plant tissues; an increase in the concentrations of metal removed from water resulted in an increase of metal concentrations in plant tissues. Also the metal removal rate depended on the type of metal in solution (0.921) but it was not really affected by the presence of the three metals together in solution (Table 6.2, Figure 6.6).

## 6.4 Conclusions and recommendations

The removal of heavy metals in water hyacinth ponds and its effect on plant biomass production were carried out in pilot scale ponds under batch flow. A

synthetic Hillman solution was used as culture medium. The synthetic medium was polluted with Cu, Fe or Zn using copper sulphate ( $\text{CuSO}_4$ ), iron II and ammonium sulphate ( $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6 \text{H}_2\text{O}$ ) and zinc sulphate ( $\text{ZnSO}_4 \cdot 7 \text{H}_2\text{O}$ ). The three metals were studied separately at 2 mg/l, 5 mg/l, 10 mg/l and 20 mg/l and mixtures of the three metals at the same concentrations were tested.

The high correlation between the metal concentration removed from the water and the metal concentration in the biomass of water hyacinth indicated not only that they can be effectively used for the removal of heavy metals from a solution of different heavy metals but confirmed that uptake by the biomass of plants was the main metal removal process in water hyacinth ponds.

The removal rate of the metals studied (Fe, Zn, Cu) fitted in a first order kinetic model with a preferential sequence  $\text{Fe} > \text{Zn} > \text{Cu}$  of accumulation of toxic elements in water hyacinth.

However, toxicity leading to growth retardation was associated with the increase of the concentrations of metals in solution. Moreover, a complete degeneration of the plants was observed when the initial concentration of Cu was 20mg/l.

On this basis, water hyacinth can be recommended for the removal of metals from aqueous solution, but care should be taken in biomass reuse due to the potential for a high rate of accumulation of toxic elements in the plant biomass. Also, further studies should be conducted to ensure the continued performance in the removal of organic matter from the water in the light of reduction in plant growth rate due to the presence of metallic pollutants.

# 7

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## ***Effect of metal accumulation by water hyacinth on ponds performances***

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### **7.1 Introduction**

Water hyacinth, a fast growing aquatic plant, have proved their efficiency in removing metals from water and accumulating them in their organism (Kay *et al.* 1984; Delgado *et al.* 1993; Soltan and Rashed 2003; Jayaweera *et al.* 2007; Zheng *et al.* 2009; Chunkao *et al.* 2012; Ibrahim *et al.* 2012; Smolyakov 2012).

Our previous work (Refer to Chapter 6) showed that water hyacinth can remove Cu, Zn and Fe from water at rates as high as 66%, 91% and 98%, respectively. The plants were able to accumulate up to 7238 mg/Kg, 5610mg/Kg and 35 465 mg/Kg for Cu, Zn and Fe, respectively in their bodies. From this previous work, a reduction in plants growth rate due to the presence of metallic pollutants has been noticed and also, a risk of release of metals in the aqueous medium by the plants from the 11th day of treatment for Zn and Fe.

Also, it has been reported that water hyacinth leaves can survive up to 6–8 weeks in water before senescence (Coetzee *et al.* 2009). This may allow plants to up-take and release metals in the medium. This situation leads to wondering about what happens to the quality of effluent from ponds with water hyacinth that have already been used to treat influent highly contaminated with metals, with this risk of releasing metals.

Also, a water hyacinth system for domestic wastewater treatment is mainly used for its ability to remove and recycle the organic load and nutrient from water (Reddy and Debusk 1984; Brix 1997; Rodriguez and Jenssen 2005; Slak *et al.* 2005; Maharjan and Ming 2012). This ability, related to their high growth rate, is what makes them in the first place attractive for wastewater purification (Jayaweera *et al.* 2007; Kutty *et al.* 2009; Yi *et al.* 2009; Nestic and Jovanovic 2010; Wang *et al.* 2012). Water hyacinth ponds have proven their efficiency to remove up to 98% of BOD, 83% of TN and 80% of phosphorus (EPA 1988; Maharjan and Ming 2012). These reported performances were obtained for ponds where the plants present in water have not been subjected to any pre-stress. But we must make clear that water hyacinth ponds in a treatment plant will face, apart from the accumulation of metals, organic loads and nutrients to be removed under the stress of metals accumulated. For this reason there is a need to study the effect of accumulated toxic elements in plants biomass on the organic matter removal.

A search of the literature showed a gap regarding the effect of the accumulated metals in plants biomass on the future development of plants and their treatment performances. The present work is carried out to assess the effect of different concentrations of metallic pollutants accumulated in plants bodies on their growth and their performances in organic load and nutrient removal. It is aiming to demonstrate that the performances of water hyacinth in organic load and nutrient removal and the effluent quality of ponds are highly influenced by the immediate history of the plants especially the amount of toxic elements such as metals in their body.

The objectives of this study were:

- ✓ to evaluate the organic and nutrients removal rates of water hyacinth with different quantity of metals in their bodies
- ✓ to analyse the influence of metals content on the plants growth rate.
- ✓ to assess the possibility of release of heavy metal in the effluent water

## **7.2 Materials and methods overview**

The experiment tried to assess the effect of metal content in plant biomass on the growth of the plants and their performances to treat wastewater. It was conducted in-field on the University Campus of Abomey-Calavi (Benin - West Africa). Anaerobically pre-treated high strength domestic wastewater used in Chapter 4 was used as influent.

Experiment was performed with fifteen (15) mini-ponds of type MP-2 as described in Chapter 3. The ponds were operated in batch flow mode with the retention time was fifteen days.

Samples of water hyacinth were collected from the previous experiment (Chapter 6) where plants were exposed previously to different concentrations of Cu, Zn or/and Fe. These plants have accumulated different concentrations of those metals in their body as demonstrated in chapter 6. Four plants of water hyacinth containing metal in their biomass as showed in Table 7.1 were introduced in each container.

The number of the plants and the fresh weight of their biomass were measured at the beginning and the end of the experiment. The average metal content of the plant materials was determined at the end of the experimental period in order to estimate the quantity of metal that was released by water hyacinth in the medium.

The treatment performance of the system was monitored by the evolution of the environmental parameters and standard measurement of COD, TSS, TNK, P- $\text{PO}_4^{3-}$  at the beginning, the seventh and fifteenth day of experiment. Each result is given as an average of three measurements taken from the same samples. The normalised methods stated in chapter 2 were used for the determination of the values of the parameters.

**Table 7. 1 : Characteristics of the previous culture medium and metal concentration in plants material**

N°	Name of the container	Initial metal concentration of the previous culture medium			Metal concentration in plants		
		Cu (mg/l)	Fe (mg/l)	Zn (mg/l)	Cu (mg/kg)	Fe (mg/kg)	Zn (mg/kg)
1	Control	-	-	-	-	-	-
2	Cu2	2	-	-	120	-	-
3	Cu5	5	-	-	799	-	-
4	Cu10	10	-	-	2 915	-	-
5	Fe2	-	2	-	-	5 858	-
6	Fe5	-	5	-	-	7 943	-
7	Fe10	-	10	-	-	13 115	-
8	Fe20	-	20	-	-	30 464	-
9	Zn2	-	-	2	-	-	390
10	Zn5	-	-	5	-	-	652
11	Zn10	-	-	10	-	-	2 469
12	Zn20	-	-	20	-	-	3 212
13	M_2	2	2	2	110	6 083	1 152
14	M-5	5	5	5	644	10 676	1 547
15	M-10	10	10	10	3 166	12 651	2 392

## 7.3 Results and discussions

### 7.3.1. Environmental parameters

Throughout the experimental period, the mean water temperature was between 23.5°C and 23.8°C with the maximum value of 24.5°C and the minimum value of 22.8°C. The temperature showed a relatively low variation in ponds. The observed values were within the optimum range for growth of water hyacinth (Allgayer 2006).

In all the ponds, an increase of the pH was observed from the beginning until the fifth day followed by a decrease up to the end of the experimental period. The pH, from the initial value of 7.2, reached values between 7.5 and 8 and then dropped to values ranging from 6.4 to 7.1 in all ponds. The increase in pH at the beginning of the experiment may be associated to the reactions occurring in the medium for the adaptation of the plants and the growth of algae observed in

ponds. After the adaptation period, the decrease of pH observed was associated to the pH adjustment by the plants of water hyacinth to its optimum range as concluded in chapter 5. The same behaviour was observed in ponds 2 and 3 during the experiment of the chapter 4.

### 7.3.2. Metal in plants and performance of Ponds

Samples of wastewater were taken from ponds and analysed for COD, TSS, TNK and phosphate to check the performances of ponds. The removal percentages were then calculated. The results are plotted in figure 7.1.

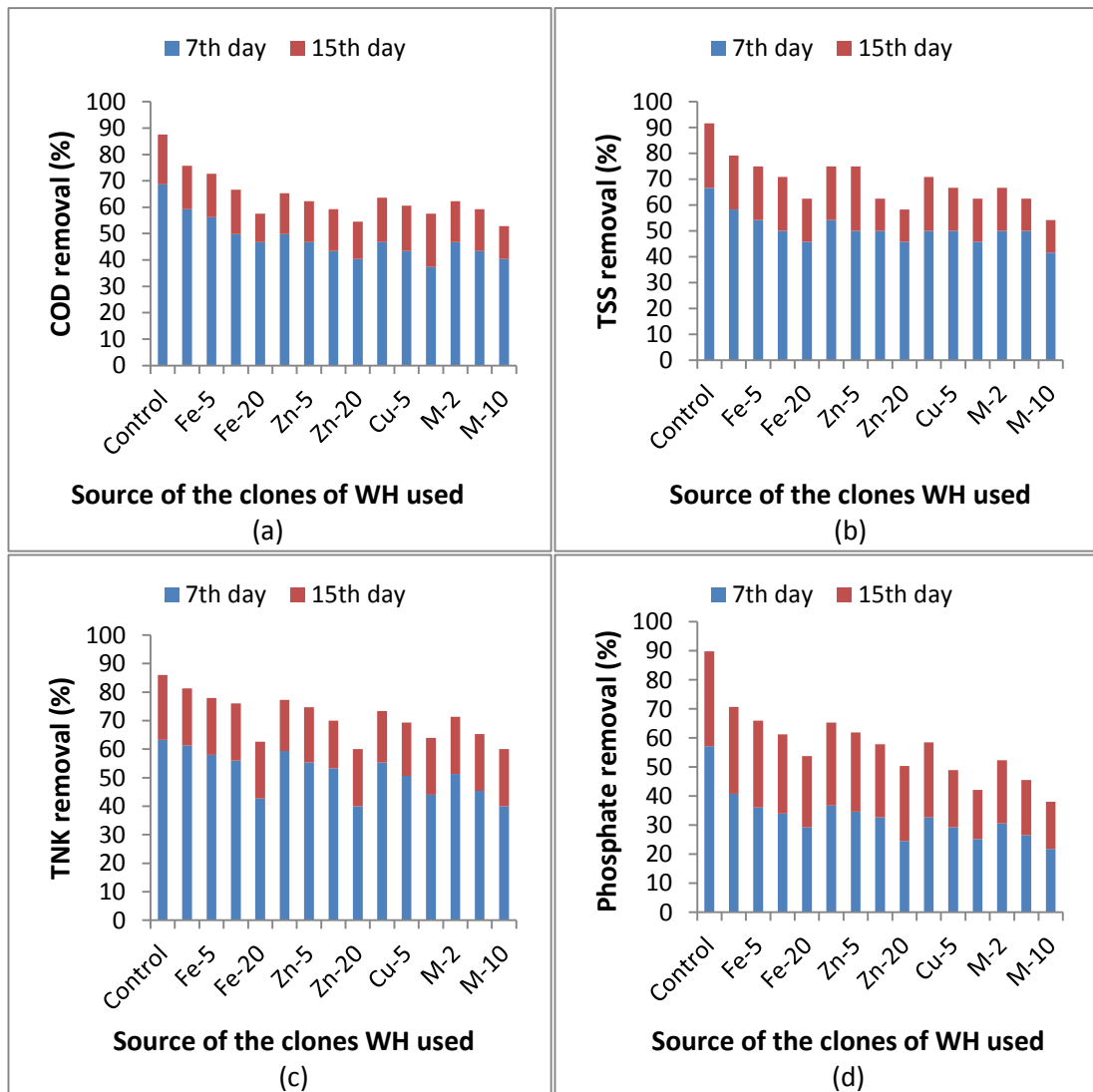


Figure 7. 1 : COD, TSS, TNK and phosphate removal rates as function metal accumulated in plants

The abscissa values (Fe-2, Fe-5 ..... ) of Figure 7.1 referred to the immediate past history of the water hyacinth plants used for the experiment. It showed the metals content of the culture medium in which the plants used for the actual experiment were grown. Table 7.1 showed for each culture medium, the metal content of the water hyacinth plants used in the actual experiment. The results will be therefore be discussed by combining the information on the Figures 7.1 to that of the Table 7.1.

The general observation of the Figure 7.1 showed that the removal rate decreased with an increase of metal content of the plants, regardless of the type of metal contained in the plants and the type of pollutants to be removed.

Indeed from Figure 7.1(a), it appeared that the removal rates of COD decreased from 76% for water hyacinth previously cultured in solution containing 2mg of Fe /l (Fe-2: water hyacinth containing 5 858mg of Fe/kg in their biomass-Table 7.1) to 58% for plants with 30 464mg of Fe/kg in their biomass (Fe-20), 65% for plants with 390 mg of Zn/kg of biomass (Zn-2) to 54% for plants with 3 212mg of Zn/kg of biomass (Zn-20) and from 64% for plants with 120 mg of Cu/kg of biomass (Cu-2) to 58% for plants with 2 915 mg of Cu/kg of biomass (Cu 10). From Figure 7.1 (b), (c) an (d) the trends for TSS, TNK and phosphate removal performances were similar to that of COD. The removal rates for ponds with water hyacinth plants having only Fe, Zn or Cu varied between 79% (Fe-2) and 63% (Cu-10), 81% (Fe-2) to 64% (Cu-10) for TNK and 71% (Fe-2) to 42% (Cu-10) for P-PO<sub>4</sub><sup>3-</sup>. Thus, it appears from the analysis of these results that the presence of Cu in the plants is more toxic and affected the performance more than of Zn or Fe.

However for all the parameters, the best performances, after fifteen days of retention, were observed in the control pond with removal rates of 92%, 88%, 86% and 90%, respectively for TSS, COD, TNK and P-PO<sub>4</sub><sup>3-</sup>. These percentages corresponded to removal rates of 17.33 mg O<sub>2</sub>/l.day for COD, 4.82 mg of TSS/l.day, 17.33 mg of TNK /l.day and 0.88 mg of P-PO<sub>4</sub><sup>3-</sup>/l.day. The lowest removal rates of 54%, 53%, 60% and 38%, respectively for TSS, COD, TNK and

P-PO<sub>4</sub><sup>3-</sup>, were obtained for the pond planted with water hyacinth plants grown in the solution containing a mixture of 10mg/l of each metal (Fe, Cu and Zn).

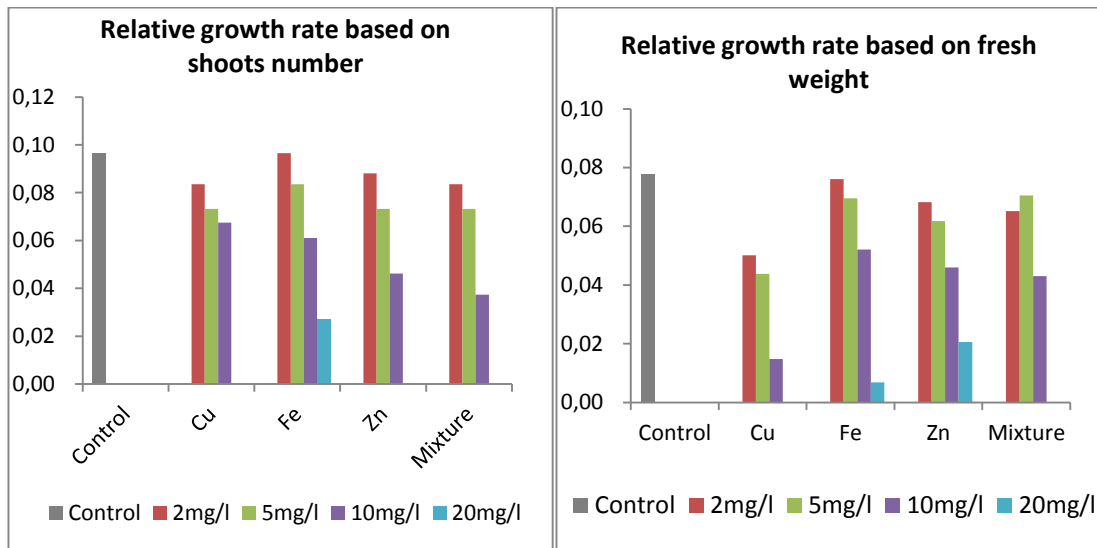
The results in Figure 7.1 clearly showed that there was considerable effect of the type and the quantity of metals contained in the plants biomass on their treatment capacity. These observations confirm the result obtained in the previous Chapter 6 and, also our suspicion that the presence of metals affected the macrophyte ponds performances in removing organic loads and nutrients.

The reduction in pond performance due to the accumulated metals in plants can be related to the reduction in plants growth rate and in their assimilation capacity. Despite the important role of Fe, Zn and Cu as trace nutrients in plant metabolism, especially Cu for plastocyanin synthesis, photosynthetic electron transport and the enzymatic oxidation of some compounds, excess of the heavy metal may be toxic to green plant (Filbin and Hough 1979; Guilizzoni 1991) (Bidwell, 1974;). In particular, Mishra et al. (2008) have pointed out an inhibitory effect of Cu on photosynthesis of water hyacinth tissue from a concentration of 2mg/l. Excess heavy metals, and copper in particular as the most toxic, may damage not only the photosynthetic apparatus but also the membrane integrity. Such a situation would affect the mechanisms by which plants will acquire organic carbon or maintain it in their organism (Filbin and Hough 1979; Guilizzoni 1991; Bidar *et al.* 2007). This damage, added to the reduction in plant growth, may explain the difference in pollutant removal showed by the present results.

It should also be mentioned that as pointed out in Chapters 4 and 5, in all the cases, the major part of the organic loads (COD) and TNK were removed in water hyacinth ponds within seven days retention time. This confirmed that seven days retention time is the optimum for organic load removal in water hyacinth ponds.

### 7.3.3. Metal in plants and their growth

The water hyacinth relative growth rates were determined based on biomass fresh weight and plant shoots number. Figure 7.2 (a) and (b) summarise the results from the calculation process.



**Figure 7. 2 :** Water hyacinth relative growth rate as function of plant source

The water hyacinth relative growth rate to the number of shoots (Figure 7.2 (a)) decreased gradually from the control pond, through the Fe ponds, followed by Zn ponds, Cu ponds to the ponds with water hyacinth shoots cultured in the medium with the mixture of the three metals.

The relative growth rate to shoots number decreased from 0.10/day to 0.03/Day for Fe ponds, 0.09/day to 0.05/day for Zn ponds, 0.08/day to 0.07/day for Cu ponds and 0.08/day to 0.04/day for the ponds with mixture of the three metals.

The best relative growth rate to plants shoots number of 0.1/day was obtained in the control pond and the pond with plants from medium with 2mg of Fe/l. However the relative growth rate to biomass weight of the pond Fe-2 was lower than that of the control ponds, which meant that the presence of the Fe in the plants body did not affect the multiplication activities but affected somewhat the increase in weight of the biomass.

Also, when looking at the relative growth rate to weight (Figure 7.2 (b)), the trend was the same, but the increase in biomass weight in the ponds with plants from mixture solutions was more pronounced than in ponds with plants cultured in medium with Cu.

Comparison of growth rate calculated here to that obtained with the artificial polluted medium (Chapter 4) showed that in general, the relative growth rates were higher with the culture of plants which have accumulated metals in wastewater than when culturing the water hyacinth in medium polluted by the metals. For example the relative growth rate to shoots number of the water hyacinth when cultured in the artificial medium polluted by 2mg/l of Cu was 0.054/day. This rate reached 0.084/day when the plants, which have accumulated Cu from this previous culture, were grown in pre-treated wastewater. This might be explained by the fact that when the culture medium is polluted by the heavy metal, the new shoots as well as the mother plants faced the stress of the presence of the metal as they were both developing in the medium containing the toxic element; while the metal accumulated in plants body affected only the mother plant as the heavy metals was not present in the medium in which they were developing. But when the metal content of the plants were very high, the metabolism activities were severely damaged and the growth rates were more affected than when in the polluted medium. This was the case of the plants from the culture media with 20mg of Fe/l, 20 mg of Zn/l or 10 mg of Cu/l.

It is clear from these results that the high accumulation of metals in water hyacinth affected their metabolism activities leading to the reduction of their growth even up to the blocking of their multiplication.

#### **7.3.4. Release of metal by plants in water**

The metal content of the water hyacinth plants used in the experiment were measured at the beginning and summarised in Table 7.1. At the end of the experiment, the metal content of the initial plants put in the medium were

determined. These initial and final metal contents were used to determine the approximate metal release rate of the plants. The obtained results were summarised in Table 7.2. The release rate calculated here did not take into account the quantity of metal that could be assimilated by the plants. As the initial metal contents of the medium was negligible and the number of mother plants was very small (four), and thus their total mass of metal release in the medium very low, no attempt to measure the quantity of the metal released in the medium by dosing the effluent was made.

**Table 7. 2 : Metal release rate by water hyacinth in water**

Concentrations of metal in the initial culture medium (mg/L)	Release rate (./day)		
	Cu	Zn	Fe
2	0.13	0.03	0.02
5	0.17	0.02	0.01
10	0.15	0.04	0.02
20		0.03	0.06
2	0.10	0.03	0.01
5	0.15	0.05	0.02
10	0.15	0.05	0.01

The release rate of Cu is higher than that of the Fe and Zn. It may be due to the fact that Cu is very toxic to the plants, it is readily realised when possible, and at rate higher than it can be accumulated in the plant. In fact, the release rates, varying between 0.10/day and 0.15/day, were higher than the removal rate obtained in chapter 4. The low release rate observed with Zn and Fe showed better assimilation of Zn and Fe by the plants. There is then more risk of pollution of the system through release when treating Cu

## 7.4 Conclusions and recommendations

Water hyacinth plants which have accumulated different concentrations of Cu, Zn or/and Fe in their body, after being cultured in artificial culture polluted by these metals, were used in mini-pond to treat anaerobically pre-treated high strength domestic wastewater.

The performance of the ponds was monitored by the evolution of environmental parameters and by standard measurement of COD, TSS, TNK, P-PO<sub>4</sub><sup>3-</sup> at the beginning, the seventh and fifteenth day of experiment. The plant relative growth rates were determined and the metal content of the plants at the end of the experiment were measured.

The pond performance and the plant relative growth decreased with an increase in metal contained in the plant bodies. The maximum removal rates of 92%, 88%, 86% and 90%, respectively for TSS, COD, TNK and P-PO<sub>4</sub><sup>3-</sup> were obtained with the control solution. The lowest removal rates of 54%, 53%, 60% and 38%, respectively for TSS, COD, TNK and P-PO<sub>4</sub><sup>3-</sup>, were obtained for the pond planted with water hyacinth plants grown in the solution containing a mixture of 10mg/l of each metal (Fe, Cu and Zn). In addition it was observed that there was a release of the metals in the treated wastewater by the plants, with higher release rate for the Cu.

This demonstrated that there is a risk of pollution of the system through release when treating Heavy metals in water hyacinth ponds; care should then be taken with the subsequent effluent of the system after the treatment of wastewater containing metal pollutions.

Also, when treating wastewater polluted by heavy metals, one should take into account the related reduction in organic loads and nutrient removal rates when predicting the quality of the effluent.

# 8

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## ***Environmental parameters in combined water hyacinth/duckweed treatment systems and algae based systems***

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### **8.1 Introduction**

Duckweed and water hyacinth ponds are attractive and cost effective solution to face wastewater management problem in developing countries, especially when combined with resource recovery and biomass reuse to generate income (Gijzen and Ikramullah 1999; Kivaisi 2001).

Water hyacinth ponds are reported very efficient in nutrient and organic loads removal (Orth and Sapkota 1988; Rodriguez and Jenssen 2005; Zhang *et al.* 2011). These ponds cannot withstand high organic loading rates over 1000 mg O<sub>2</sub>/l of BOD<sub>5</sub> (Maharjan and Ming 2012). However, limitations which have been reported in literature for water hyacinth ponds and observed in our previous chapters are their low level of pathogen removal and the acidic quality of their effluent, regardless the influent pH condition, which do not allow for a direct discharge in the nature or reuse of the effluent in agriculture (Polprasert *et al.* 1992; Kim and Kim 2000; Maharjan and Ming 2012).

On the other hand, the high pathogen removal capacity reported for duckweed ponds made them good candidates for polishing in a treatment system (Awuah 2006). Also, the reported high oxygen content of the effluent allowed for reuse in

aquaculture (Caicedo Bejarano 2005; Awuah 2006). Yet, duckweed is reported as very sensitive to high organic loads with the related ammonia toxicity which can lead to a complete degeneration of the plants (Reed *et al.* 1995; Zimmo *et al.* 2005) This makes them not appropriate to be used as first ponds for the treatment of high or medium strength wastewater.

In attempt to overcome the effect of the high strength wastewater generated in developing countries on duckweed ponds systems and to have a polished effluent that can be reused from our macrophyte based system, we have proposed a combined treatment water hyacinth-duckweed system for wastewater treatment and resource recovery, where water hyacinth ponds (WHP) were put ahead of duckweed ponds (DWP). The WHP will reduce greatly the organic load on the DWP but may also change environmental and physicochemical characteristics.

The biological and physicochemical processes in a conventional waste stabilisation pond (WSP) are very complex and influenced by many factors including environmental conditions (Metcalf and Eddy 1995). The presence of macrophytes covering ponds and the combination in a system of different macrophytes introduces even more complexity to the system because of interactions between plants and water, the differences in plant behaviour and the possible reduction of sunlight penetration. This may greatly influence the system effluent water quality, especially as changes occur in the influent wastewater environmental quality. It is then important to have a good understanding of these environmental conditions prevailing in the system.

The environmental conditions in WSP systems are well documented; but for macrophytes ponds, especially such a combined system, they have not been studied in detail. The objective of this study is therefore to:

- make a comparison between the environmental conditions within a combined macrophyte pond systems and a standard WSP and to,

- investigate the stability of effluent environmental quality as changes occur in influent quality.

## **8.2 Materials and methods overview**

The experiment was conducted in pilot plant constructed on the University Campus of Abomey-Calavi, Benin in a tropical climate. The system was to treat the wastewater from the University halls as described in Chapter 3. The System was operated at a total flow rate of 6 m<sup>3</sup>/day.

Intensive monitoring environmental parameters (temperature, pH, conductivity, TDS, e<sub>H</sub>) was carried out as described in 3.4.3 of Chapter 3.

The longitudinal evolution of the measured parameters was compared by plotting, for a specific measuring hour, their mean values at each point of the system over the experimental period. The analysis of relationship between the parameter changes, the type of pond, the time of the day, the position of the pond in the system and the distance from the inlet of the system was done by a Principal Components Analysis (PCA) of Pearson type. The set of data used in the analysis contained 99 observations for each variable of a set of nine variables. Each observation represented the mean value of eight days of measurements. The variables were:

- the time of the day (PD) represented by 10, 14 and 17, respectively for 9 to 10 a.m., 13 to 14 (p.m). and 17 to 18 (p.m).;
- the type of pond (TP), represented by 0, 1, 2, and 3 respectively for the influent, the WSP, WHP and DWP;
- the distance from the system inlet (DS), expressed in metres;
- the position of the pond (PP) in the system, represented by 0, 1, 2, 3 and 4 respectively for the influent, the first ponds, the second ponds, the third ponds and the fourth pond;
- the environmental parameters: pH, temperature (T), TDS, conductivity ( $\chi$ ) and the redox potential e<sub>H</sub>.

The variations in the environmental quality of the influent and effluent of each pond were compared using Boxplots and ANOVA.

### 8.3 Results and discussion

The observed results are presented on Figure 8.1. The correlation circle of the PCA presented in Figure 8.2 and the correlation factors in Table 8.1 were made to refine the observations.

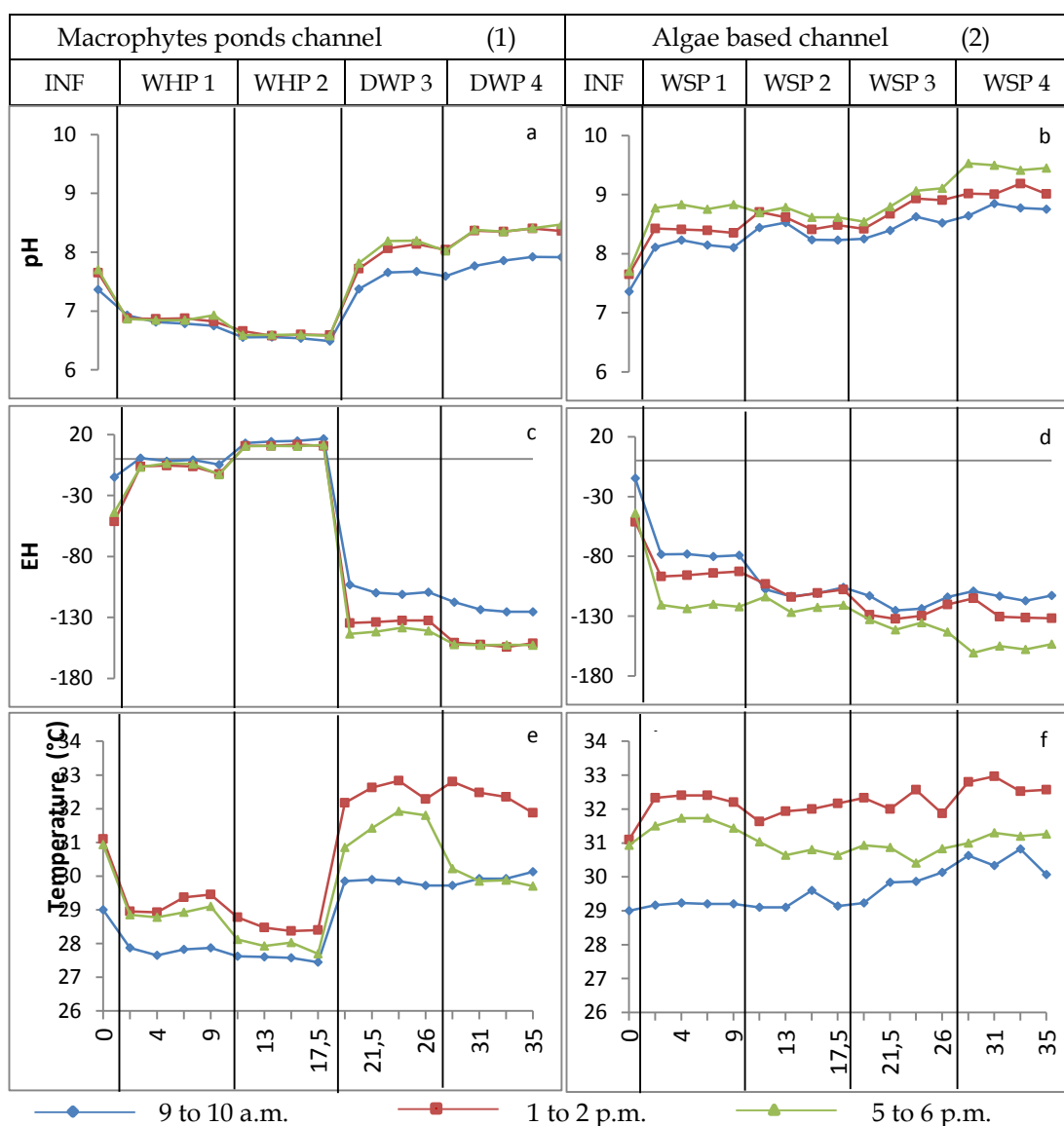


Figure 8. 1 : Changes in pH,  $e_H$  and temperature along the treatment channels

The longitudinal profiles established for the morning (9 to 10 a.m.), the afternoon (1 to 2 p.m.) and the evening (5 to 6 p.m). are represented on Figure 8.2 for pH,  $e_H$  and temperature and Figure 8.3 for TDS and conductivity.

The factorial plan F1 and F2 showed that more than 80% of the total variance was expressed (Figure 8.2). They demonstrate a good representation and distribution of the variables studied; then the analysis of the data in this plan is acceptable. The first axis F1, which explains about 57.96% of the total variance, was expressed in its negative poles by the position of the pond (PP), the distance from the inlet of the system (DS), the temperature (T) and the pH, and in its positive pole by the conductivity ( $\chi$ ), the total dissolved solids (TDS) and the redox potential ( $e_H$ ). The second axis F2, is constituted by the type of pond (TP) in its positive direction, the temperature (T), the pH and the period of the day the measurements were taken (PD) in its negative direction (Figure 8.1). The correlation factors are presented in Table 8.1.

The mean values of the system influent pH varied from 7.4 and 7.7. In the macrophyte based system, these values of pH drop to values around 6.9 at the entrance of the WHP 1 and decrease gradually to reach stable values between 6.5 and 6.7 in WHP 2 (Figure 8.1-a).

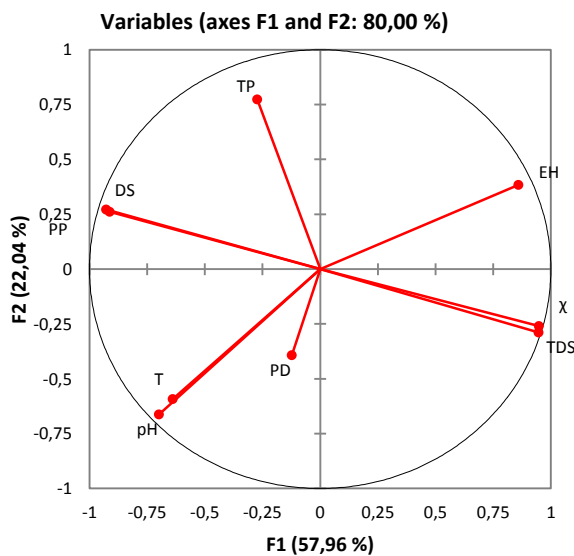


Figure 8. 2 : PCA correlation circle

**Table 8. 1 : Correlation matrix (Pearson (n))**

Variables	DS	TP	PP	PD	T	pH	$\chi$	TDS	e <sub>H</sub>
DS	<b>1</b>	<b>0.335</b>	<b>0.967</b>	0.000	<b>0.358</b>	<b>0.476</b>	<b>-0.913</b>	<b>-0.931</b>	<b>-0.634</b>
TP	<b>0.335</b>	<b>1</b>	<b>0.361</b>	0.000	-0.123	<b>-0.394</b>	<b>-0.444</b>	<b>-0.455</b>	-0.013
PP	<b>0.967</b>	<b>0.361</b>	<b>1</b>	0.000	<b>0.364</b>	<b>0.483</b>	<b>-0.941</b>	<b>-0.959</b>	<b>-0.652</b>
PD	0.000	0.000	0.000	<b>1</b>	<b>0.336</b>	0.195	-0.015	-0.011	-0.168
T	<b>0.358</b>	-0.123	<b>0.364</b>	<b>0.336</b>	<b>1</b>	<b>0.751</b>	<b>-0.460</b>	<b>-0.424</b>	<b>-0.763</b>
pH	<b>0.476</b>	<b>-0.394</b>	<b>0.483</b>	0.195	<b>0.751</b>	<b>1</b>	<b>-0.484</b>	<b>-0.467</b>	<b>-0.891</b>
$\chi$	<b>-0.913</b>	<b>-0.444</b>	<b>-0.941</b>	-0.015	<b>-0.460</b>	<b>-0.484</b>	<b>1</b>	<b>0.992</b>	<b>0.696</b>
TDS	<b>-0.931</b>	<b>-0.455</b>	<b>-0.959</b>	-0.011	<b>-0.424</b>	<b>-0.467</b>	<b>0.992</b>	<b>1</b>	<b>0.686</b>
e <sub>H</sub>	<b>-0.634</b>	-0.013	<b>-0.652</b>	-0.168	<b>-0.763</b>	<b>-0.891</b>	<b>0.696</b>	<b>0.686</b>	<b>1</b>

Values in bold are different from 0 with a significance level  $\alpha=0.05$

This observation was in accordance with my previous result obtained with mini-ponds in Chapter 5 stating that water hyacinth can adjust the water pH to its requirement and that the optimum range of pH for WHP was 6.4 to 7.1. It also confirmed the acidic condition reported in WHP in several previous papers (Kim and Kim 2000).

The mean values of pH from WHP2 had a sharp rise to mean values between 7.4 and 7.8 at the entrance of the DWP1 followed by a gradual increase to stabilise at values between 7.8 and 8.5 (Figure 8.1-a). This shows that, as demonstrated for water hyacinth in Chapter 3, duckweed plants have also the ability to adjust the pH of the medium to their requirement. DWP can then be used effectively to adjust the pH of the acidic effluent from WHP to requirements for reuse in agriculture and aquaculture.

The pH value measured in all the four WSP and the two DWP showed that alkaline conditions were prevailing in the two types of ponds; even though the pH values of the WSP (maximum mean value 9.5) were higher than those in the DWP (maximum mean value 8.5); The pH value of the DWP were most of the time close to the neutral value (Figure 8.3-a and b). Similar pH conditions were reported by several researchers for WSP and DWP in full scale studies as well as pilot and laboratory scale studies (Caicedo *et al.* 2000; Al-Nozaily 2001; Awuah *et al.* 2004; Caicedo Bejarano 2005).

The variation of pH over the day showed a clear difference between WSP, DWP and WHP. The gradients in pH over the day were more remarkable in the WSP and DWP than in the WHP. Also, the changes in pH seemed gradual in the WSP while the gradients between the afternoon and the evening in the DWP were not significant. The variation of pH throughout the day in the WHPs was negligible. However, in all the ponds the highest mean values of pH were obtained in the evening and the lowest ones in the morning (Figure 8.1-a and b). However, from the reading of the covariance circle (Figure 8.2), just a slight negative effect of the period of the day on the pH was observed. This may be due to the effect on the covariance factor of the low variation of the pH in WHP ponds throughout the day and in DWP in the afternoon.

The variation of the pH-values during the day can be explained by the fact that from morning to evening, the accelerated photosynthesis activities of the plants present in the ponds (algae, water hyacinth, duckweed) consumed the CO<sub>2</sub> at a higher rate than the production rate of the bacteria, resulting in an increase of the pH. This explains the observation of the highest values in the evening. On the contrary, during the night, in the absence of sunlight, the respiration of the plants led to the production of CO<sub>2</sub> which, added to that produced by the bacteria, resulted in a drop of the pH and then the observed low pH-values in the morning.

As the water hyacinth have more parts in air, it seems that the CO<sub>2</sub> and the O<sub>2</sub> exchanges were mainly with the surrounding air rather than the water; this may explain the little variation of pH observed in those ponds. Such a situation is susceptible to affecting the O<sub>2</sub> level in ponds since the presence of the plant may impede also the entry of the O<sub>2</sub>, like that of the sunlight, from the air at the water surface.

Figure 8.2-c shows an increase of the redox potential from the influent to the WHP. The potential continued increasing up to the outlet of WHP2 and then dropped sharply in DWP1. It continued decreasing gradually until the outlet of

DWP 4. In the WSP system (Figure 8.1-d),  $e_H$  decreased gradually up to the end of the system. Water hyacinth seemed to have a tendency to reduce the medium while duckweed and algae tend to make the medium more oxidising. This observation is in accordance with the anoxic conditions reported for WHPs (less than 2.1 mg O<sub>2</sub>/l) and the aerobic conditions in WSPs (over 16.6 mg O<sub>2</sub>/l) and DWP (up to 6 mg O<sub>2</sub>/l) (Orth and Sapkota 1988; Zimmo *et al.* 2000; Awuah *et al.* 2004; Lu *et al.* 2008; Ansa *et al.* 2012).

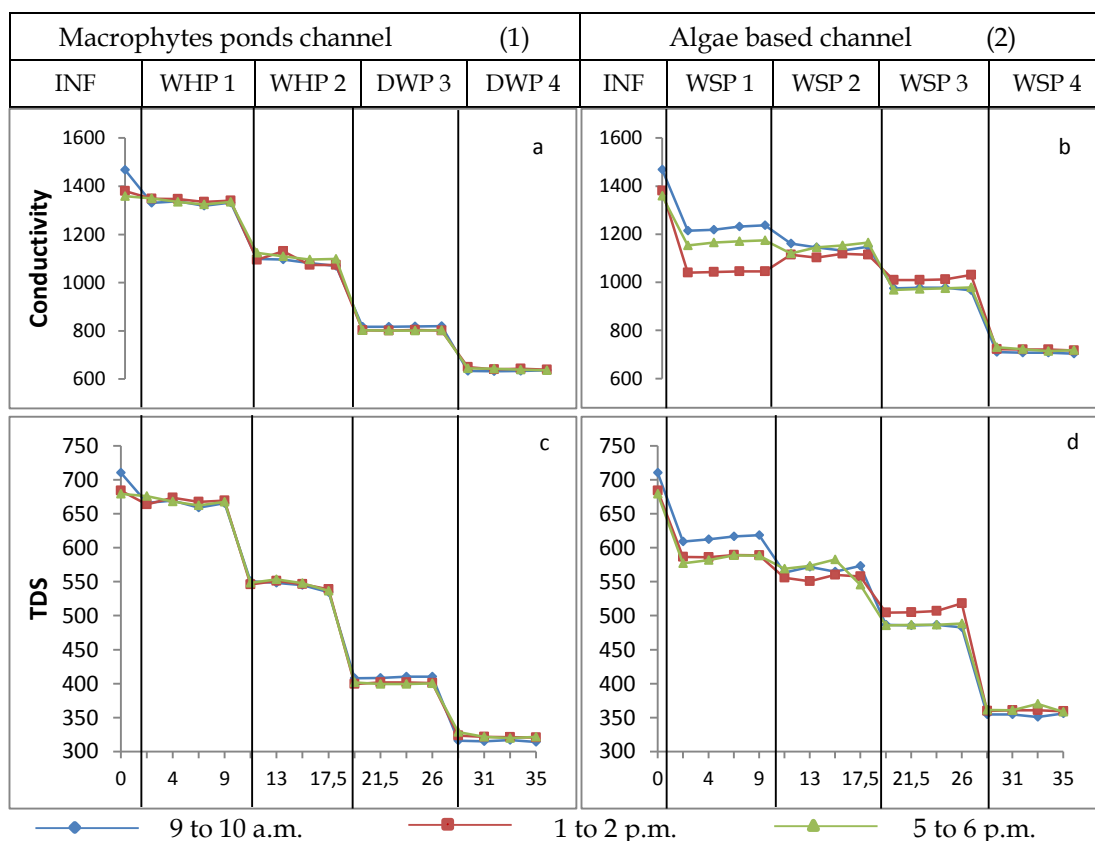
Longitudinal profiles did not show great variations of temperature within a pond when compared for the same period of the day. In general the highest temperature is observed in all the ponds between 1 and 2 p.m.; this was associated with the sunlight that is usually observed in this period of time in the area. The lowest temperatures were observed with the morning measurements taken between 9 and 10 a.m. (Figure 8.1-e and f). So, the temperature depended on the period of the day at which the measurements were taken but not on the distance from the inlet of the system. This is confirmed by the high level of significance ( $\alpha > 0.05$ ) of the correlation between the temperature and period of the day (Table 8.1). Nevertheless, a slight difference in the behaviour of the temperature was noticed in DWP4. Low temperatures were measured in the evening; this was due to the presence of some trees at this side of the system which covered the pond by their shade from the afternoon, reducing the sunlight penetration in DWP4.

An insignificant negative relation between the temperature and the type of ponds was expressed through the determination of the correlation factor which was -0.123 (Table 8.1). However, it can be observed from Figure 8.2-e that there was a drop in temperature from the influent to the WHP. The mean temperatures remained lower in the two WHPs (between 27.5°C and 29.5°C) compared with the DWP and the WSP. The difference in mean temperatures between the DWP (29.7 to 32.8) and WSP (29.0 to 32.6) during the experimental period was not really remarkable (Figure 8.2-e and f). The low temperature observed in WHPs was associated with the low penetration of sunlight due the

water hyacinth coverage; but the effect of presence of duckweed covering ponds surface on sunlight penetration seemed less important. The intensity of sunlight penetration in macrophyte ponds depends then on the type of macrophytes.

With the high values of pH and temperature and the oxidising conditions in the DWPs and WSPs, a good removal of microorganisms can be; indeed several studies have referred to them as the main factors influencing pathogen removal in ponds (Curtis *et al.* 1992).

A decrease of the TDS and the conductivity from mean values was observed along the two treatment systems (Figure 8.3). The mean values of TDS passed 677 - 711 mg/l to respectively 314 - 323mg/l and 356 - 413 mg/l at the outlet of macrophyte based system and the algae based system.



**Figure 8. 3:** Changes in TDS and conductivity along the treatment channels

The reading from the correlation circle (Figure 8.1) revealed that the first dimension F1 seemed to oppose the TDS and conductivity to the distance from

the inlet and the position of the pond. Also, from Table 8.1 it appears that there is a significant negative correlation between the TDS, the conductivity and the type of ponds. No significant effect of period of the day on these parameters was observed. These observations confirm that the removal of TDS increased with the total length of the system and the number of ponds in series. The difference observed in the effluent quality and the negative correlation with the type of ponds proved that the presence of macrophytes in ponds improves the effluent TDS quality. The decrease of TD and conductivity along the system is indicative of a reduction of pollutants along the two systems.

From the covariance circle, it appears that all the environmental parameters were in significant correlation with the temperature (Tale 8.1), confirming that the temperature is a factor controlling ponds performance.

To visualise the variation of effluent quality of the ponds, a boxplot is presented in Figure 8.4. The boxplot shows a summary of a distribution of the parameters.

From the first reading of the boxplots, it appears in general that, in all the ponds and for all the parameters, the medians of the distributions were not centred in the boxes; they differ from the mean values even though the median of the  $e_H$  in WHP1 and WHP2 and the pH of the effluent of the DWP4 seemed very close to the mean values. The plots were asymmetric.

The increase in pH from WSP to WSP can clearly be observed on the pH plots. The drop of the pH in the WHP and the low pH gradients of the effluent from these ponds are clearly shown.

The effluent temperature from the macrophyte system seemed to be in the same range as the influent temperature. The effluent from WSP has a higher temperature even though the differences is not too large (about  $0.5^{\circ}\text{C}$ ). Also the gradient of the effluent temperature from the different WSPs in the system is higher compared with the macrophyte pond in the same position. WHP2 has the lowest temperature gradient.

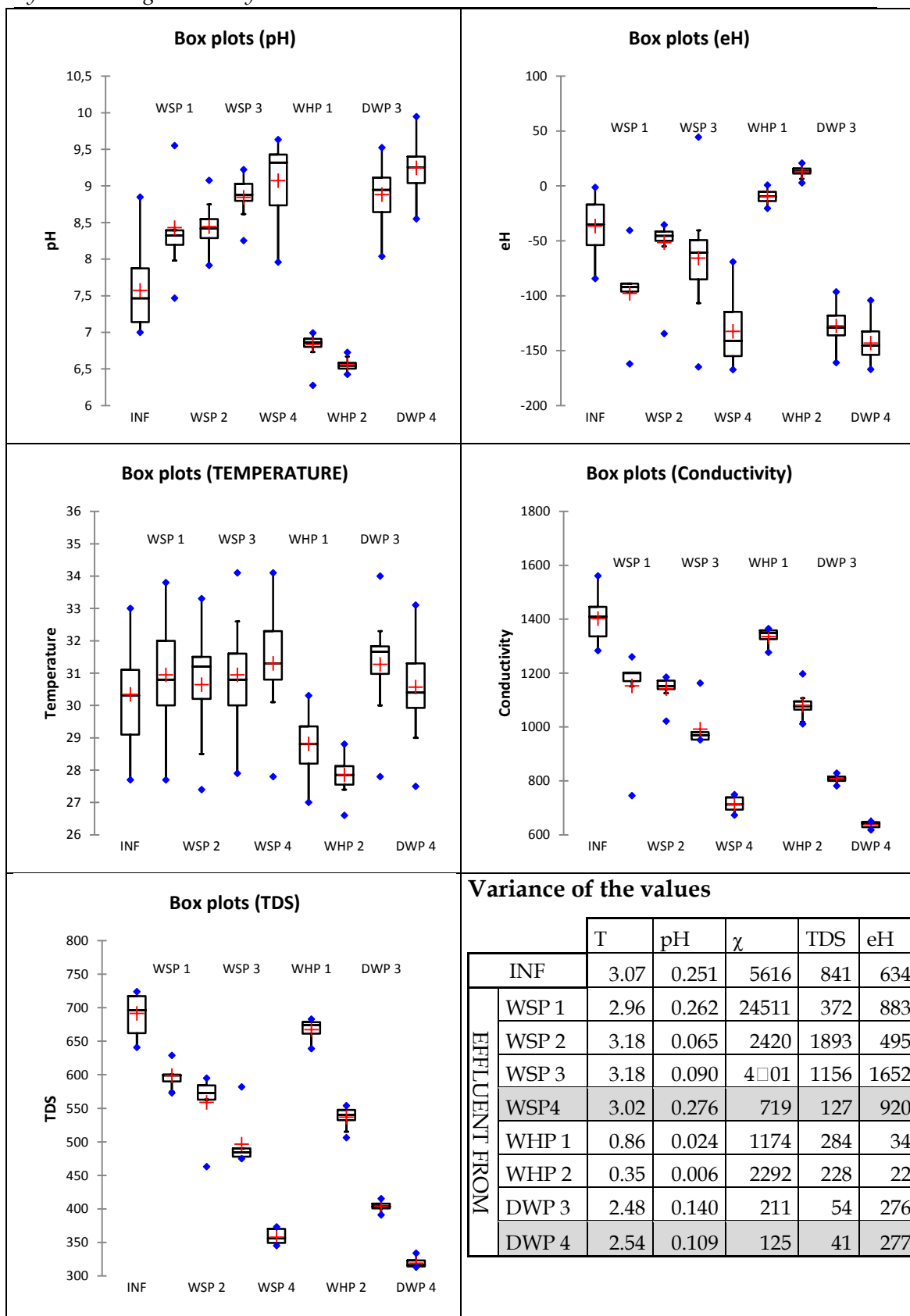


Figure 8. 4 : Changes in environmental conditions of the influent and effluent of ponds

The interquartile spacings of the boxes representing the different parameters measured are larger for the effluent of the WSP4 than that of the DWP4 (Figure 8.4). Also, the lengths of most of the plots' whiskers are greater for WSP4 than DWP4. This is confirmed by the high values of variance of the distributions which were, for the algae based system respectively, 3.02, 0.276, 719, 127, and 920 for T, pH, conductivity, TDS, and  $e_H$  and for the macrophyte based system, of 2.54, 0.109, 125, 41 and 277 respectively for T, pH, conductivity, TDS, and  $e_H$  distributions of the effluent (Figure 8.4).

This shows that the environmental quality of the effluent of the macrophyte based system was less affected by the changes in the influent wastewater environmental quality than the WSP system. Nevertheless, for the two systems, the variation in the effluent TDS content is less pronounced than that of the influent.

## **8.4 Conclusions and recommendations**

The experiment was conducted in a pilot plant treating domestic wastewater in tropical climate. In order to compare the environmental conditions within a combined WHP and DWP system and a WSP system and to investigate the effect of the fluctuating influent wastewater environmental quality on the stability of effluents quality, two channels of treatment were used under the same operating conditions; one was composed of four WSPs and the second line consisted of two WHPs followed by two DWPs.

Different environmental conditions prevailed in the ponds. It appeared that acidic and reducing conditions with low temperature were observed in WHPs, while alkaline and oxidising conditions with high water temperature prevailed in the DWPs and WSPs. However, the pH values for DWP were closer to neutral values and the temperature in the DWPs was slightly lower than that in the WSP system.

Also, in all the ponds the lowest pH and temperature were measured in the morning. The highest values of pH were observed in the evenings while the highest values of the temperature were measured in the afternoon.

A decrease of TDS and conductivity was observed in the two systems as the distance from the inlet increased. In all the cases, comparing the removal at equivalent points in the two systems, the removal observed was higher in the macrophyte based system than that in the algae based system.

A comparison of variances of the environment values of the effluent of the two systems showed that the macrophyte system was less affected by the influent quality change than the algae based system.

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## **System performance variability under fluctuating loading rates in combined water hyacinth/Duckweed ponds and algae based ponds**

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### **9.1 Introduction**

Wastewater characteristics vary greatly from area to area depending on the health status and the nutritional habits of the population, the water usage pattern but also the wastewater collection systems (Mara 1977; Metcalf and Eddy 2003) At the same time, changes can be observed in these characteristics throughout the year at the same area or even during a day, due to the difference in habits, changes in water usage pattern over the time and the presence of several wastewater collection systems in the same area. This is especially true for developing world cities where different wastewater collection systems coexist, leading to high variation in influent wastewater loading to treatment plants (Koné 2002; Koanda 2006; Hounkpè *et al.* 2014).

On the other hand, several researchers have demonstrated that the different treatment processes occurring in ponds can be highly affected by the pollutant loading rates (Zimmo *et al.* 2002; Caicedo Bejarano 2005; Awuah 2006). WSPs are reported to be able to treat several types of wastewater when designed for it (Mara 1987; SEIDL *et al.* 2003; Awuah 2006). Water hyacinth ponds (WHPs) were able to treat wastewater with organic loading rate greater than 1000 mg/l of

BOD<sub>5</sub> (Maharjan and Ming 2012). As for duckweed ponds (DWP), several studies have recommended them for the treatment of secondary effluent with COD less than 530 mg/l or nitrogen concentrations less than 100 mg/l (Mandi 1994; Zimmo *et al.* 2005). However, the changes in effluent qualities of these pond systems in relation to the fluctuation of the influent loading rate were not evaluated, although these different types of ponds have been monitored successfully in general for different loading rates (Orth and Sapkota 1988; Alaerts *et al.* 1996; Kim and Kim 2000; Al-Nozaily 2001; Zimmo *et al.* 2002; Caicedo Bejarano 2005). Most of the research so far has been performed using a specific treatment line with a single type of pond and mostly at laboratory or pilot scale. No attempt to combine ponds, for instance WHP and DWP, in the same treatment line has been seen in the literature.

In the process of technology development, the assessment of treatment efficiencies of such technologies must be evaluated under an expected fluctuating loading before its utilisation on a larger scale. The aim of this study was, then, to compare the overall performance between a combined macrophyte-based channel and a WSP channel and to evaluate the effect of the fluctuating pollutant loading rates on the channels effluents.

## **9.2 Materials and methods overview**

The study was conducted using a full-scale pilot plant set up at the University of Abomey-Calavi (UAC), Benin in a tropical climate. The system consisted of two parallel treatment channels as described in Chapter 3.

Performance of the two channels was monitored for a 62 week period at different flow rates (6 m<sup>3</sup>/day; 12 m<sup>3</sup>/day; 4.5 m<sup>3</sup>/day; 2 m<sup>3</sup>/day; 4 m<sup>3</sup>/day and 8 m<sup>3</sup>/day) as presented in Chapter 3.

On-site measurements were done and samples were taken regularly at 10 different points in the system as presented in Chapter 3 for the determination of the parameters used for performance evaluation.

The measured parameters for performance evaluation were chemical oxygen demand (COD), total suspended solids (TSS), biochemical oxygen demand (BOD<sub>5</sub>), orthophosphate, nitrate, nitrite, total organic nitrogen (TN), total Kjeldahl nitrogen (TKN) and faecal coliforms (CF). They were measured in liquid samples collected on average twice a month.

Additional parameters such as dissolved oxygen (DO), temperature, pH, conductivity ( $\chi$ ) and total dissolved solids (TDS) were also monitored.

After 12 weeks of experiment, fish culturing started.

## **9.3 Results and discussions**

### **9.3.1. Environmental parameters**

Throughout the experimental period, the influent wastewater pH varied between 6.01 and 7.98. Acidic conditions were always observed in the WHP; alkaline conditions prevailed in the DWP and WSP. The effluent pH from the WSP channel varied between 7.92 and 9.61, while it ranged between 7.22 and 9.07 for the WHP/DWP channel. Dissolved oxygen (DO) ranged between 3.8 mg/l and 5.6 mg/l for the effluent from the WHP/DWP channel and 4.9 mg/l and 8.2 mg/l in the effluent from the WSP channel. The lowest values of DO were always observed in the WHP/DWP channel. Temperatures ranged from 25.9°C to 34.9 °C for all the ponds. The observed temperatures in the ponds were highly related to the ambient temperature. The lowest temperatures for each measurement were observed in the WHP while the highest temperatures were measured in the WSP. TDS were least in the effluent of the macrophyte based system. These conditions were the same as the ones observed and discussed in the previous chapter.

### **9.3.2. Comparative effect of fluctuating loading rate on the combined WHP/DWP and WSP**

During the 62 weeks of experiment, WHP/DWP channel and WSP channel were monitored at different hydraulic flow rates (HFRs) and different TSS, COD, TN, PO<sub>4</sub><sup>3-</sup> and faecal coliform average loading rates, as presented in Table 9.1 and Table 9.2. HFR varied between 2 m<sup>3</sup>/day and 12 m<sup>3</sup>/day. The organic loading rate varied between 228 mg/l and 803 mg/l of COD or between 1311 mg/day and 5563 mg/day for each channel (Table 9.1).

A comparison of the effect of hydraulic and pollutant loading rates on the experimental channels, with and without macrophytes, was carried out.

Table 9.1 and Table 9.2 present the average removal rate of pollutants over the experimental periods. Figure 9.1 represents the influent TSS, COD, TN and phosphate loads and the effluent quality of each channel as a function of the HFR. The variance of the effluent quality parameters of the two channels is presented in Table 9.3.

During operation, it was observed that on the first days after a change of HFR and influent wastewater quality (Table 9.1 and Table 9.2), the effluent quality from ponds were affected by the previous operational values. The removal rate of pollutant was initially low immediately after a change from a high loading rate to a low loading rate, but was gradually enhanced during the first week. Removals became considerably more stable and gradually increased with increasing operating time with the same HFR and influent quality. The length of the stabilisation period depended on level of the change. For example, by changing from 26.32x10<sup>9</sup> of FC/day (HFR = 12 m<sup>3</sup>/day) to 3.78x10<sup>9</sup>/day (HFR = 4.5 m<sup>3</sup>/day), the FC content of effluents was very high the first days before dropping after two weeks of treatment; this affected the average value of FC in the effluent computed for the period where the HFR was 4.5 m<sup>3</sup>/day from WHP/DWP.

**Table 9. 1 : Channels loading rate and removal rate of TSS, COD and TN over the experimental period**

Parameters		Unit	Week							
			0	24	30	36	42	48	56	62
<b>Total HFR</b>		<b>m<sup>3</sup>/day</b>	<b>6</b>	<b>12</b>	<b>4.5</b>	<b>2</b>	<b>6</b>	<b>4</b>	<b>8</b>	
TSS	Influent load	mg/l	212	132	123	226	135	191	105	
		g/ day	1272	1580	555	451	810	766	842	
	Removed from WHP/DWP	g/ day	626	671	236	212	373	364	368	
		%	98.4%	85.0%	85.0%	94.0%	92.0%	95.1%	87.3%	
	Removed from WSP	g/ day	516	426	181	174	284	313	290	
		%	81.1%	54.0%	65.0%	77.0%	70.0%	81.7%	68.7%	
COD	Influent load	mg/l	593	464	291	705	228	803	328	
		g/ day	3558	5563	1311	1410	1367	3212	2621	
	Removed from WHP/DWP	g/ day	1657	2309	506	639	593	1543	1118	
		%	93.1%	83.0%	77.2%	90.7%	86.7%	96.1%	85.3%	
	Removed from WSP	g/ day	1447	1641	446	551	472	1370	926	
		%	81.3%	59.0%	68.0%	78.2%	69.0%	85.3%	70.7%	
TN	Influent load	mg/l	156.0	173.6	86.4	204.0	83.3	195.0	129.9	
		g/ day	936.0	2083.2	388.8	408.0	499.8	780.0	1038.8	
	Removed from WHP/DWP	g/ day	401.4	881.2	144.1	176.3	181.9	333.3	427.0	
		%	85.8%	84.6%	74.1%	86.4%	72.8%	85.5%	82.2%	
	Removed from WSP	g/ day	374.8	717.5	118.2	179.5	135.9	328.3	372.8	
		%	80.1%	68.9%	60.8%	88.0%	54.4%	84.2%	71.8%	

**Table 9. 2:** Channels loading rate and removal rate of phosphate and faecal coliforms over the experimental period

Parameters		Unit	Week						
			0	24	30	36	42	48	56
<b>Total HFR</b>		<b>m<sup>3</sup>/day</b>	<b>6</b>	<b>12</b>	<b>4.5</b>	<b>2</b>	<b>6</b>	<b>4</b>	<b>8</b>
PO <sub>4</sub> <sup>3-</sup>	Influent load	mg/l	34.4	36.8	28.4	28.3	27.8	61.7	27.8
		g/day	206.1	441.6	127.8	56.6	167.0	246.9	222.2
	Removed from WHP/DWP	g/day	94.7	167.8	50.5	26.1	73.5	116.8	80.9
		%	91.9%	76.0%	79.0%	92.0%	88.0%	94.6%	72.8%
	Removed from WSP	g/day	73.2	130.3	41.5	19.8	58.5	97.5	61.5
		%	71.0%	59.0%	65.0%	70.0%	70.0%	78.9%	55.3%
FC	Influent load	10 <sup>6</sup> /l	2.21	2.14	0.84	4.05	2.03	0.98	2.24
		10 <sup>9</sup> /day	13.29	25.62	3.78	8.10	12.18	3.92	17.92
	Removed from WHP/DWP	10 <sup>9</sup> /day	6.64	12.79	1.89	4.05	6.09	1.96	8.96
		Unit Log	3.98	2.88	2.93	4.05	3.88	3.16	3.42
	Removed from WSP	10 <sup>9</sup> /day	6.64	12.77	1.89	4.05	6.09	1.96	8.96
		Unit Log <sub>10</sub>	5.08	2.49	3.16	4.58	4.49	3.59	3.27

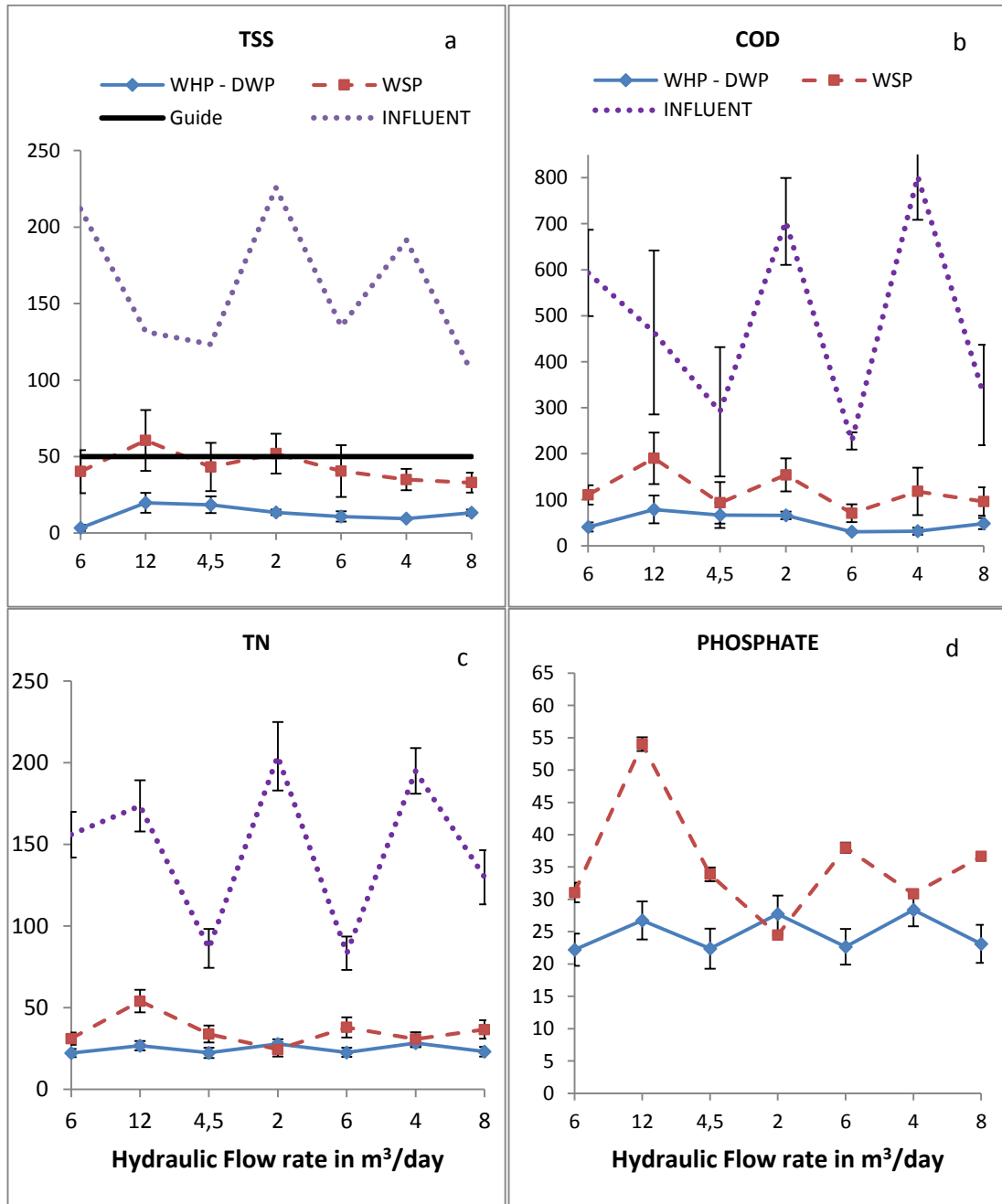
The hydraulic flow rate (HFR) of each channel is the half of the total HFR presented in Table 9.1 and Table 9.2.

The values presented in Table 9.1 and Table 9.2 are the average values computed for each HFR over the period of time it flowed.

The system design HFR was 6 m<sup>3</sup>/day.

Log<sub>10</sub> removal is determined by Log<sub>10</sub>(Influent FC/Effluent FC) = - Log<sub>10</sub>(1-removal percentage)

This re-adaptation period and this effect on the effluent quality observed after change in the loading rate are important factors to take into account in the design and the prediction of the performance of pond systems working under highly fluctuating loading.



Guide = required standard limit for discharged or reuse

**Figure 9. 1:** Influent and effluent TSS, COD, TN and phosphate as function of HFR

In general, removal efficiencies of the WHP/DWP channel fluctuated between 85.0% and 98.4% for TSS, 73.2% and 96.1% for COD, 72.8 and 86.4% for TN and 72.8% and 94.6% for phosphate over the experimental for the different HFR (Table 9.1 and Table 9.2).

The average TSS, COD, TN and phosphate removal efficiency in the WSP channel changed from 54.0% and 81.7%, 59.0% and 85.3%, 54.4% and 88.0%, 55.3% and 78.9%, respectively (Table 9.1 and Table 9.2).

In almost all the cases, removed quantities of TSS, COD, TN and phosphate were higher for the WHP/DWP channel than for the WSP channel (Table 9.1, Table 9.2 and Figure 9.1) with subsequent better effluent quality in WHP/DWP channel compared with that of the WSP channel.

This result showed that the macrophyte based system was still more efficient than WSP system in removing TSS, organic load and nutrient even under fluctuating loading rate, this improved efficiency having been demonstrated for constant loading rate by several studies (McDonald and Wolverton 1980; Orth and Sapkota 1988; Zimmo *et al.* 2002; Awuah *et al.* 2004; Caicedo Bejarano 2005).

In general, from Figure 9.1 it can be seen that for the four parameters TSS, COD, TN and phosphate, the effluent water quality depended on the influent pollutant loading. For instance, for a total influent load of 555 g/day and 1 311 g/day of TSS and COD, respectively, total TSS removed was 417 g/day (236 g/day plus 181 g/day) for the two channels and total COD removal of 952 g/day (506 g/day plus 446 g/day) was achieved. With an increase of the loading rates to 1580 g/day and 5563 g/day TSS and COD, respectively the total TSS removal rates reached 1 097 g/day (671 g/day plus 426 g/day) for TSS and 3 950 g/day (2309 g/day plus 1641 g/day) (Table 9.1, Table 9.2 and Figure 9.1). The correlation test also confirmed that the daily quantity of the pollutants discharged through the effluent of the two channels was highly related to the total loading rate of the pollutants in the influent wastewater at significance level alpha equal 0.05.

However, the effluent water quality from the WHP/DWP channel was more stable with very small changes compared to the effluent from the WSP channel. This was shown by the low values of the standard deviation, the variation of quality parameters values observed on the curve (Figure 9.1) and the variance of these quality parameters (Table 9.3). In fact, the effluent of the WHP/DWP channel had TSS, COD, TN and phosphate content up to average values of 19.7 mg/l, 79 mg/l, 28 mg/l and 8.8 mg/l, respectively (Figure 9.1), with variance of 37 mg/l, 528 mg/l, 12mg/l and 8 mg/l, respectively (Table 9.3). The observed ranges for the WSP channel were up to 61 mg/l for TSS, 190 mg/l for COD, 54 mg/l for TN and 15.1 mg/l for phosphate (Figure 9.1) with greater variance of this parameters compared to that of the effluent from WHP/DWP channel (Table 9.3). Also, the TSS content of the effluent from the WSP channel was most of the time higher than the required value of 50 mg/l for reuse (WHO 2006).

These results indicate that there was no significant inhibition effect on the WHP/DWP effluent quality related to the fluctuation of the loading rates, and a combined WHP/DWP can be operated with good effluent quality with changing loading rate, as regards to TSS, organic loads and nutrient. This may be explained by the fact that macrophytes easily adapt their nutrient up-take to the medium quality. Also, their roots, acting as a filter, may greatly contribute to the stability of the quality of the effluent. A combined WHP/DWP system can therefore be seen as an appropriate system for treatment plants working under fluctuating loading rate.

**Table 9. 3:** *Variance of the effluent quality parameters of WHP/DWP and WSP channels*

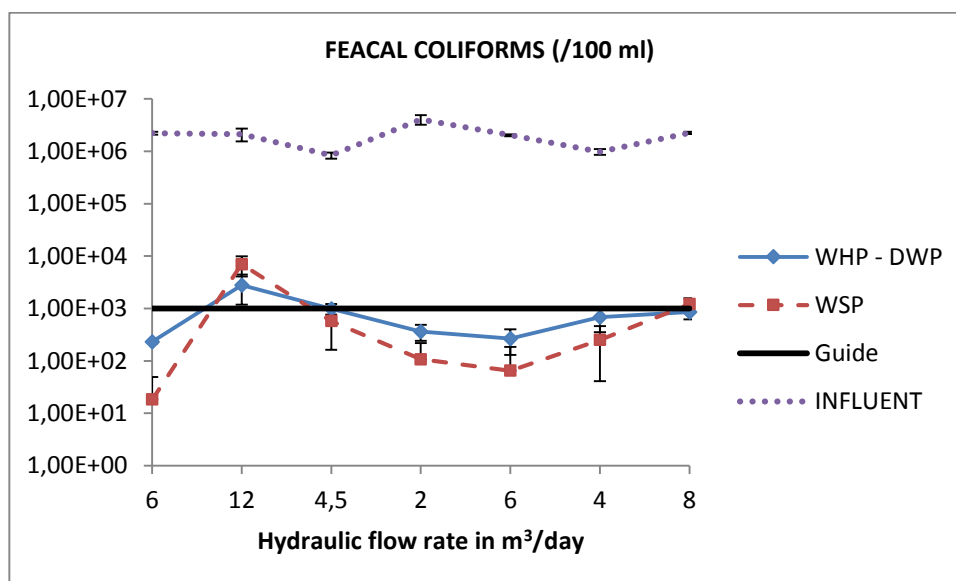
Effluent from	Variance				
	TSS	COD	TN	PO <sub>4</sub> <sup>3-</sup>	FC
WHP/DWP channel	37	528	12	8	1.01E+06
WSP Channel	223	2 567	97	14	6.61E+06

Another observation, from the correlation analysis, is that the nutrient and the faecal coliform removal in the WSP channel was highly related to the influent

HFR and the nutrient concentration of the influent at a level of significance alpha equal 0.05. An increase of the concentration of nutrient and FC in the effluent was observed with an increase in the HFR. For instance, with an influent TN concentration of 86.5 mg/l and 83.3 mg/l respectively at rates of 4.5 m<sup>3</sup>/day and 6 m<sup>3</sup>/day, the effluent from the WSP channel had a TN content of 24.5 mg/l and 38 mg/l, respectively (Figure 9.1-c and d).

In the WHP/DWP channel, only the faecal coliform removal seemed to be related to the inflow at level of significance alpha greater than or equal to 0.05.

In fact the faecal coliform removal in the WHP/DWP channel was very low at a HFR of 12 m<sup>3</sup>/day. The average value of the faecal coliforms in the effluent was 3 920 /l, which was far greater than the required value for reuse (Figure 9.2). In general, the average values of faecal coliforms in the effluent of the WHP/DWP channel ranged between 230 /l and 2800 /l.



**Figure 9. 2:** Microbial quality of the influent and the effluent as function of hydraulic flow rate

The effluent quality of the WSP channel, with regard to the faecal coliform content, had average values ranging between 18/l and 6875/l; it was most of the time better than that of the WHP/DWP channel. This is in accordance with some results obtained with some studies under normal loading rate (not fluctuating)

((Zimmo *et al.* 2004; Caicedo Bejarano 2005; Awuah 2006). However, it was noticed that at high hydraulic flow rates (12 m<sup>3</sup>/day for instance), the faecal coliform removal rate was better in the macrophyte based channel than in the WSP channel. Also, the effluent from WHP/DWP quality, with regard to FC, was more stable with a lower variance than that of the WSP channel (Table 9.3). This is in accordance with the behaviour observed above with the other quality parameters measured.

Based on the completely mixed conditions in the ponds, the average first order die-off constant of the faecal coliforms  $K_T$  can be determined by modelling the die-off of faecal coliforms in each pond as a first order kinetic equation (Marais 1974; Metcalf and Eddy 2003):

$$\frac{N_{if}}{N_{ef}} = 1 + K_T \theta \quad (9.1)$$

Where  $N_{if}$  is the number of faecal coliforms per 100 ml of the influent wastewater,  $N_{ef}$  is the number of faecal coliforms per 100 ml of the effluent wastewater and  $\theta$  is the retention time (which depends on the HFR).

For each channel, by taking into account the five ponds (anaerobic pond + 2 WHP + 2 DWP for the first channel, and anaerobic pond + 4 WSP for the second channel), the equation for each channel became:

$$\frac{N_{if}}{N_{ef}} = (1 + K_T \theta_a)(1 + K_T \theta_1)(1 + K_T \theta_2)(1 + K_T \theta_3)(1 + K_T \theta_4) \quad (9.2)$$

$\theta_a$ ,  $\theta_1$ ,  $\theta_2$ ,  $\theta_3$  and  $\theta_4$  are the retention time of the anaerobic pond, WHP1 or WSP1, WHP2 or WSP2, DWP3 or WSP3 and DWP4 or WSP4, respectively. The retention time is determined based on the actual HFR.

The results for the two channels are shown in table 9.4.

In the WHP/DWP channel, the average  $K$  values fluctuated between 0.36/day and 1.11/day while they varied between 0.48/day and 1.88/day for the WSP (Table 9.4).

Zimmo, et al (2005) have reported K-values ranging from 0.17/day to 1.79/day depending on the organic loading rate for a WSP treatment system. A higher die-off constant of 2.7/day was reported by Awuah, *et al* (2004) for algae ponds. Caicedo (2005) reported a die-of constant of 0.32/day for these ponds. These differences may be the result of several factors such as the difference in retention time, the depth of the ponds and the size of ponds, which may affect the hydraulic performance (creating dead zones, short circuiting). In fact Awuah, *et al* (2004) used much smaller ponds of 0.145m<sup>2</sup> cross section and seven days retention time; the ponds used by Zimmo, *et al* (2005) had a cross-section of 3 m<sup>2</sup> and seven days retention time while the pond used by Caicedo (2005) had a cross section of 65 m x 4.95 m with 11.5 days as retention time.

**Table 9. 4:** First order faecal coliforms die-off values for the two channels

HFR (m <sup>3</sup> /day)	Total FC Loading		K-value	
	10 <sup>5</sup> /l	10 <sup>8</sup> /day	WHP/DWP	WSP
2	40.5	81.0	0.36	0.48
4	9.8	39.2	0.44	0.56
4.5	8.4	37.8	0.43	0.49
6	22.1	132.9	1.05	1.88
6	20.3	121.8	1.00	1.38
8	22.4	179.2	1.02	0.93
12	21.35	256.2	1.11	0.86

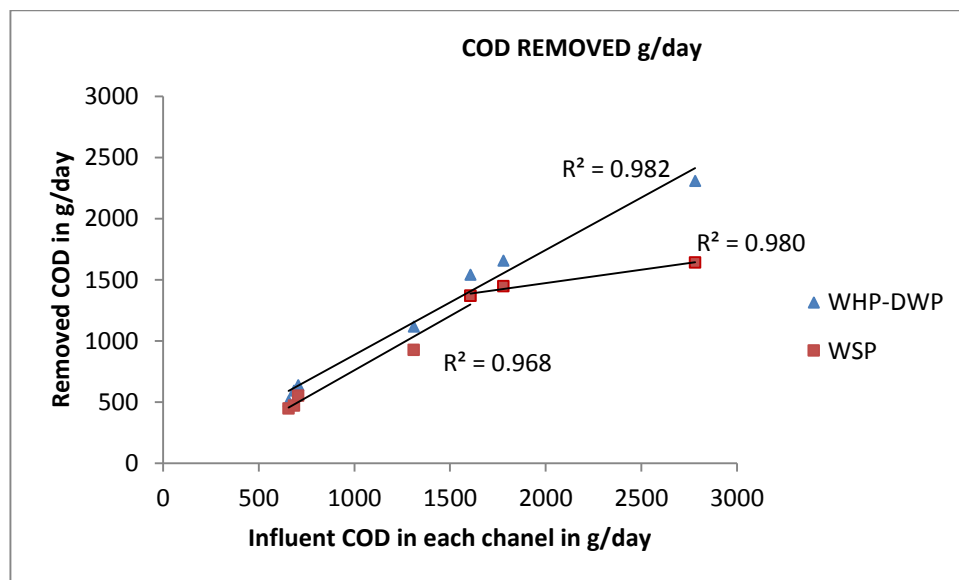
The faecal coliform profiles observed in this study (Figure 9.2 and Table 9.4) showed that lower faecal coliform removal might not always be encountered in the macrophyte based ponds compared with the WSP contrary to what was reported by several studies (McDonald and Wolverton 1980; Orth and Sapkota 1988; Polprasert *et al.* 1992; Zimmo *et al.* 2005; Awuah 2006; Maharjan and Ming 2012)

In fact, at a lower HFR than the design HFR, the K-values were greater in the WSP channel, confirming that the faecal coliform die-off was better in that

channel than in the macrophyte based channel. For example, at a HFR of 2 m<sup>3</sup>/day, the K-value was 0.36 and 0.48 for WHP/DWP channel and WSP channel, respectively. These K-values observed at low HFR represented, for each channel, the lowest value over the experimental period. This is associated with the long retention time applied with low hydraulic flow rate which seemed not to add too much to the faecal coliform removal in ponds.

On the contrary, during higher HFR (Table 9.4), the K-values of the WHP-WSP channel were better than that of the WSP channel. This is in accordance with the faecal coliform counts of the effluent from the two channels (Figure 9.2). The low K-values encountered in the WSP may be explained by the fact that high inflow did not allow for sufficient retention time for coliform die-off in the ponds, but in the macrophyte ponds, faecal coliform attachment to the area offered by plants may have played an important role in reducing the removal as stated by some authors (Brix 1994; Bonomo *et al.* 1997; Awuah 2006).

It can be observed that none of the two channels performed very well as regard to the microbial quality of their effluents, especially at high HFR.



**Figure 9.3 :** COD removed from each channel as function of influent COD

Figure 9.3 shows the quantity of organic pollutant removed as a function of the influent COD.

The organic load removed increased with the influent daily load for both the channels. The COD removed was always higher in the WHP/DWP than that in the WSP channel despite the low oxygen level in the ponds of this channel compared to the WSP channel.

The increase in the organic load removed was regular and continuous with an increase in daily loading rate for the WHP/DWP channel. The removal rate of COD was found to be linearly related to the influent load. This yielded, with a correlation value  $R^2$  greater than 0.98, the following equation (Figure 9.3):

$$\lambda_r = 0.857 \lambda_v + 29 \quad (9.3)$$

This indicated that, in macrophyte ponds the organic load removal was not proportional to the available oxygen in the ponds. The main mechanism of reduction of COD in the macrophyte ponds was therefore the reduction in TSS through biomass attachment due to presence of plants in ponds (Brix 1994; Bonomo *et al.* 1997). In fact a significant correlation ( $\alpha = 0.05$ ) was observed between the TSS removed and COD removed in the WHP/DWP channel.

In the WSP channel, the COD removal rate was faster at lower loading rate than at the design loading. In the two cases, the removed organic load was found to be linearly related to the influent loading with correlation values greater than 0.96. At loading rates smaller than 1606 g/day, the removal kinetic coefficient was 0.887; it dropped to 0.218 when the influent loading was greater than 1606 g/day. This result confirmed that the organic load removal was highly affected by the influent loading rate in the WSP channel; this was not the case of the macrophyte based system. This result meant that in the WSP treatment system, the removal performance (percentage) will be highly affected by a fluctuation of the loading rate. A sudden increase in the loading rate will lead to a high drop in the removal percentage; care should then be taken when predicting the effluent quality.

So, in general, the fluctuation of loading rates has less impact on macrophyte based system than the WSP system. The macrophyte based system seems therefore more appropriate in such operational conditions.

### **9.3.3. Performance comparison of the combined WHP/DWP channel and WSP channel**

The first experimental period of 24 weeks was used to compare the performances of the ponds of the two channels under the total design flowrate of 6 m<sup>3</sup>/day.

#### **Dissolved oxygen (DO)**

An increase of the DO was observed in the effluent of the two systems (Figure 9.4-a). The influent DO increased from an average of 1.2mg/l to an average of 7.8 mg/l at the effluent of the WSP channel and 4.2 mg/l in the effluent of the WHP/DWP channel. However, it has to be pointed out that the DO, which increased from 1.2 mg/l to 1.4 mg/l in the anaerobic pond, dropped to an average value of 1.1 mg/l after passing through the first water hyacinth pond (WHP). A slight increase in DO was observed with the second WHP. The main contribution to the DO of the effluent of the WHP/DWP channel was from the DWP. In the WSP channel, a continuous increase of DO was observed from the influent to the last pond.

The low values of DO in the WHP can be explained by the fact that the water hyacinths, with their dense emergent part, seemed to exchange the O<sub>2</sub> produced by respiration with the surrounding air than the water; in addition, their presence does not facilitate the entry of the O<sub>2</sub> from the air into the water. Similar low values of DO in water hyacinth ponds, ranging from 0.3 mg/l to 2.1 mg/l, were reported by several researchers (McDonald and Wolverton 1980; Orth and Sapkota 1988; Lu *et al.* 2008).

McDonald and Wolverton reported similar decrease in DO values, as observed in this study in WHP-1, after a 3-year study conducted on an existing, one-cell,

facultative pond in south Mississippi (1980). In their study the DO decreased from an average value of 1.5 mg/l to 0.8 mg/l in the effluent water with 100% coverage of water hyacinth.

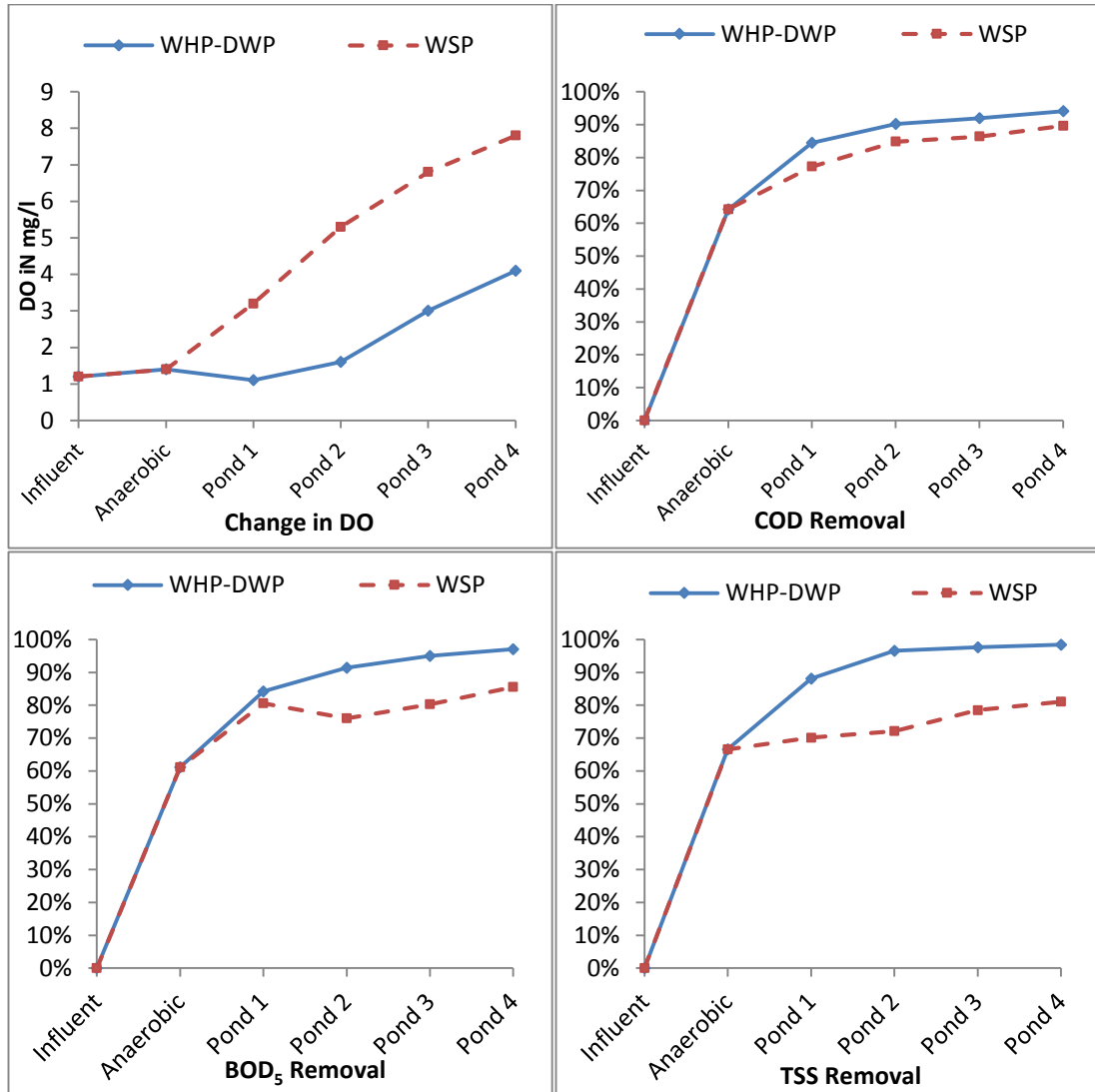
High values of DO observed in the WSP channel compared to the macrophyte-based system were reported by several researchers (Orth and Sapkota 1988; Zimmo *et al.* 2004; Awuah 2006; Ansa *et al.* 2012). In fact, the concentration of DO in the WSP channel effluent of up to 16.8 mg/l was observed by Zimmo *et al.* (2005) and Ansa *et al.* (2012); Awuah *et al.*, meanwhile, observed a DO up to 20 mg/l from an algae pond system (2006). But a lower DO concentration of 3.5 mg/l to 4.4 mg/l has been observed with a single-cell WSP system (McDonald and Wolverton 1980; Orth and Sapkota 1988; Lu *et al.* 2008). The large variation of DO from WSP based systems observed in all these studies and the present result can be explained by the difference in the number of ponds in series, the depth, the size of the systems and the hour of the day the measurements were taken, as it has been demonstrated that the oxygen content of the water increases while moving along a series of ponds and also depends on the time of the day and the depth of the measurement (Caicedo Bejarano 2005; Awuah 2006; Ansa *et al.* 2012).

The level of DO in the effluent from each of the two systems in the present study was above the limit of 4mg/l required for reuse (Jönsson *et al.* 2004; WHO 2006). However the influent of the fish pond of the macrophyte based system did not always comply with this guideline. The presence of fishes in the last pond did not seem to affect the increase of DO in its effluent. However, the density of fishes in the pond was low in this experiment; also there no other DWP working under the same condition, without fish culturing, for comparative study, to really enable the evaluation of effect of the presence of fish on DO in the pond.

### ***Organic load and suspended solids***

The organic loads and the suspended solids removal rate are presented in figure 9.4.

Most of the influent COD, BOD<sub>5</sub> and TSS were removed in the anaerobic pond. In fact the anaerobic pond removed 64%, 61% and 67%, respectively of the influent COD, BOD<sub>5</sub> and TSS. However, those removal percentages were lower than that observed in our previous set up used for the experiments discussed in Chapters 4 and 5. This observation may be due to the fact that in the previous set up, the flow condition was batch, which allows for settling without any disturbance from incoming flow and the size of the pond which was quite small.



**Figure 9. 4:** DO, COD, BOD<sub>5</sub> and TSS removal rates evolution along the two channel

Figure 9.4 -b, c and d- show that the removal rate achieved for the COD, BOD<sub>5</sub> and TSS was always higher in the WHP/DWP channel than in the WSP channel.

The overall best percentages of removal of 94%, 97% and 98%, respectively for COD, BOD<sub>5</sub> and TSS were observed with the macrophyte based channel. A higher rate of removal of TSS was observed in the WHP compared to the DWP. In fact, the two WHP 1 and WHP 2 were able to remove 96% of the TSS received from the anaerobic pond while the DWP 3 and DWP 4 removed only 53% of the TSS received from the WHP 2, giving the channel the overall high level of TSS removal of 98%.

In the WSP channel, an increase in the BOD<sub>5</sub> from an average value of 56.7 mg/l to 70.1 mg/l was observed in the WSP 2; the percentage of BOD<sub>5</sub> removed reduced from 81% to 76% in this pond. However, the overall removal in the channel was positive. The influent COD, BOD<sub>5</sub> and TSS of  $593 \pm 94$  mg/l,  $292 \pm 43$  mg/l and  $212 \pm 31$  mg/l, respectively were reduced to  $111 \pm 21$  mg/l,  $57 \pm 18$  mg/l and  $40 \pm 14$  mg /l, respectively, in the WSP channel.

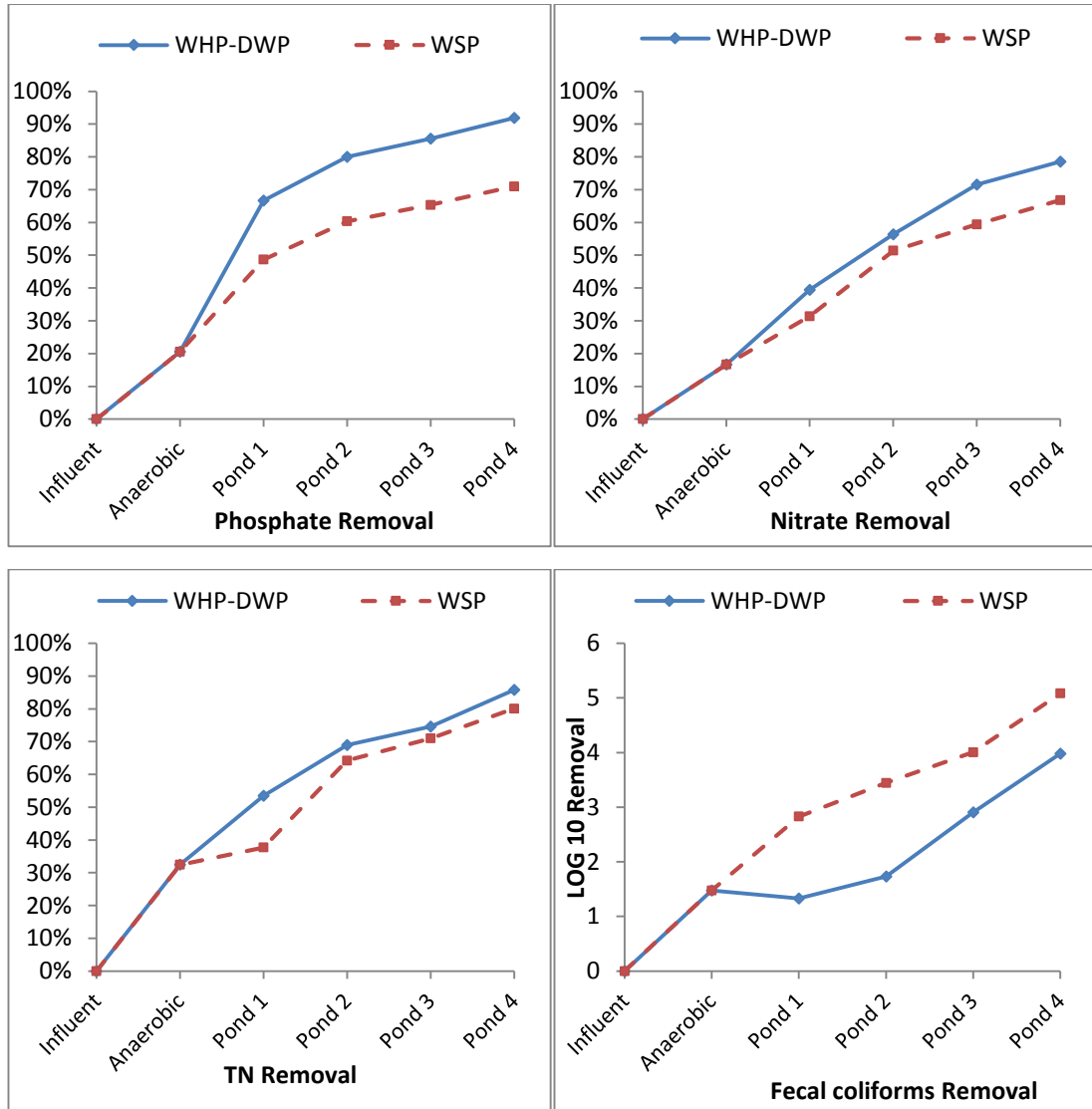
The influent BOD<sub>5</sub>/COD ratio of 0.49 increased to 0.54 at the outlet of the anaerobic pond. A continuous decrease of this ratio was observed from the first to the last macrophyte pond, with a final average value of 0.25 in the effluent water. In the algae based system, BOD<sub>5</sub>/COD ratio decreased from 0.54 to 0.49 between the inlet and the outlet of the first pond, WSP 1; subsequent increase and decrease were observed from pond to pond in the channel, with the effluent water having 0.51 as BOD<sub>5</sub>/COD ratio. This ratio is noticeably higher than that of the influent wastewater.

These results show that the effluent of the macrophyte system contained less biodegradable organic matter. These were removed by the plants. Macrophytes effected a significant removal of BOD<sub>5</sub> by directly absorbing it for their metabolisms. The high BOD<sub>5</sub>/COD observed in the algae based channel was due to the high amount of suspended algae normally carried by the effluent of those systems (Mara 1996). This could also explain the increase of BOD<sub>5</sub> observed in WSP 2. For this reason, better organic load removal in algae based

systems can be expected by filtering effluent water to reduce its algae content (Mara 1996; Zimmo *et al.* 2002).

The high TSS removal observed in WHPs can be associated with the extensive root system of the plants which provides a large surface area for pollutant attachment and also acts as a filter medium (Brix 1994; Bonomo *et al.* 1997).

**Nutrients**



**Figure 9. 5 :** Phosphate, Nitrate, TN and faecal coliform removal in WHP/DWP channel and WSP channel

The nutrient removal percentages are presented on Figure 9.5 a, b and c. The influent phosphate level of  $34.4 \pm 6.5$  mg/l was reduced by 92% and 71%, respectively in the macrophyte and algae based systems.

Total nitrogen level was reduced by 86% and 80%, respectively in the WHP/DWP channel and WSP channel. Similarly, nitrate was reduced by 79% and 67% respectively.

TN reduction in a WSP system was reported to be 46.6% by Caicedo (2005) and 77% by Zimmo, et al (2004). There is quite a difference in the present removal rate and the various reported removal rates. However, It has been reported that nitrogen removal in WSP systems can vary from a negligible rate to higher rate depending on the configuration of ponds and their operation characteristics (Silva *et al.* 1995).

### ***Faecal coliforms***

The  $\log_{10}$  removal of the faecal coliforms is presented in Figure 9.5-d.

The  $\log_{10}$  removals were respectively, 5.1 and 4.0, for the effluent of the WSP channel and WHP-WDP channel. The influent faecal coliform level was reduced in the anaerobic pond by unit  $\log_{10}$  of 1.5. A continuing increase in the faecal coliforms removed was observed in the WSP channel from the first to the last pond.

In the WHP/DWP, a decrease of the  $\log_{10}$  removal was observed in the pond WHP 1. The  $\log_{10}$  removal dropped from 1.5 to 1.3. A slight increase was observed in the pond WHP 2 where the  $\log_{10}$  removal reached 1.7. The major contribution in faecal coliform removal in the macrophyte based channel was achieved by the duckweed ponds DWP 1 and DWP 2.

The removal rate of faecal coliform observed in the WSP system conform with the reported value of up to 5 units  $\log_{10}$  by several studies ((Zimmo *et al.* 2004; Caicedo Bejarano 2005; Awuah 2006).

The overall removal of faecal coliforms in the macrophyte ponds was lower than that reported in the literature for a series of duckweed ponds (Caicedo Bejarano 2005; Awuah 2006). This was associated with the presence of water hyacinth ponds in the system, the contribution to faecal coliform removal of which is less 0.15 unit  $\log_{10}$ . The increase in the faecal coliform number in WHP 1 was in accordance with our previous observation with the mini-ponds reported in Chapter 5.

However, the faecal coliform content of effluents from the third pond of each channel (DWP3 and WSP 3) and the final effluents from the two channels, when ponds were operated at the design HFR of 6 m<sup>3</sup>/day, complied with the WHO guidelines for reuse in aquaculture and agriculture which is less than 1000 FC/l (WHO 1989; WHO 2006).

#### **9.3.4. Aquaculture and composting experiments**

The fish species Tilapia was grown in the last pond of each channel. Four fish of approximately five to six centimetres in length were put in each of the ponds. The growth of the fish was planned to be monitored by counting the number of fish and weighing them periodically. No additional input of nutrient was made in the fish ponds.

During the experiments, it was difficult to control and track the number of fish. However, with a net it was possible to catch a higher number than the initial number of fish in the Duckweed fish pond. Most of the fish were far bigger than the initial ones with length up to 10 cm (Figure 9.6). It was observed that the presence of the fish in the ponds did not affect the treatment process.

In the algae fish pond, no fish were caught. The absence of fish in this pond may be attributed to the low level of nutrient in the pond and the high diurnal variation of DO and the pH in the pond. The pH value was most of the time greater than the recommended value (6.5 to 8) required for reuse in aquaculture to avoid stress on fishes and reduce mosquito breeding (WHO 2006).



**Figure 9. 6 :** *Fish caught from the duckweed fish pond*

An alternative method should be found to monitor fish growth in ponds. Further research should be carried out to look at the public health safety and nutritional composition of the fish grown in the ponds. However the fact that tilapia are not bottom dwellers means they are less susceptible to uptake of certain contaminants that settle in sludge. Therefore, the safety may be optimized with their growth in wastewater treatment maturation ponds.

Also no mosquito breeding was observed in the ponds.

The harvested plants, especially water hyacinths, from ponds were used to optimize the composting of domestic solid waste on the experiment site. The composting experiment was not directly part of this thesis work.

## **9.4 Conclusions and recommendations**

The study was carried out as a comparative analysis of fluctuating loading rate on WSP treatment and water hyacinth/duckweed (WHP/DWP) based treatment. A pilot plant consisting of two channels, one with WSPs and the second WHP/DWPs, were operated simultaneously for 62 weeks. The system was fed with different quality of domestic wastewater at hydraulic flow rates ranging from 2 to 12 m<sup>3</sup>/day.

At the design flow rate, the WHP/DWP showed better removal of TSS, organic load and nutrient than the WSP. The DO content and the faecal coliform removal were better in the WSP compared to WHP/DWP. The  $\log_{10}$  removal of faecal coliform were 5.1 and 4.0 and the DO was 7.8 mg/l and 4.2 mg/l, respectively, for WSP and WHP/DWP. The contribution of the WHP to DO and faecal coliform removal was very low and affected the overall removal in this channel. However these ponds have contributed significantly to the TSS and organic load removal. The effluents of the two channels were found to comply with the guidelines for reuse.

Under the fluctuating loading, the average effluent TSS and COD fluctuated respectively between 33 mg/l and 61 mg/l and 71 mg/l and 190 mg/l for the WSP and from 3 and 20 mg/l and 26 and 79 mg/l, respectively, for the WHP/DWP. The effluent quality with regard to nutrient and organic loads was always better in the WHP/DWP effluent than the WSP effluent.

COD removed was linearly correlated to the influent COD with the same slope for all the loadings. For the WSP channel, two stages of the linearity existed; a slope of 0.887 was observed at low loading rate and lower slope of 0.218 was obtained for high loading rate.

The faecal coliform die-off constant fluctuated between 0.36/day and 1.11/day for the WHP/DWP channel and between 0.48/day and 1.88/day for the WSP channel. At design or lower hydraulic flow rate, the faecal coliform removal was better in the WSP than in the WHP/DWP. At high hydraulic flow rate, the removal rate of the WHP/DWP was better.

The fluctuation of the loading rates was found to have a significant effect on the WSP channel performance and effluent quality. High variation in effluent quality with regard to TSS, organic load, nutrient and faecal coliforms content was observed.

In general, the WHP/DWP channel is able to withstand fluctuating influent wastewater quality and hydraulic flow rate, leading to satisfactory performance and more stable effluent water quality than WSP channel.

Fish were able to grow in the last pond of the WHP/DWP.

The WHP/DWP is therefore more appropriate to work under fluctuating loading rate than WSP systems.

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## ***General conclusions and design guidelines***

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### **10.1 General conclusion**

The general awareness of natural resources depletion and the promotion of the integrated resources management call for the use of macrophytes, in particular floating macrophyte, ponds system as a good alternative to treat and recycle wastewater especially in tropical climate conditions. However experiences have shown that no single floating macrophyte system is able to overcome the high strength influent wastewater quality usually observed in developing countries and release, at the same time, an effluent quality meeting the requirement for reuse.

An integrated macrophyte pond system combining water hyacinth ponds and duckweed ponds is therefore proposed to take advantage on the best characteristics of each macrophyte. However the removal mechanisms and the effect of different operational conditions on treatment processes and resources recovery ability of the system need to be understood.

Therefore, it is necessary to examine the effectiveness of treatment and resources recovery under the effect of different operational conditions, such as pH, light intensity, influent metal content and fluctuating pollutants loading rate on the ponds performance and recycling ability.

Experiments were conducted in water hyacinth mini ponds and in a full scale pilot treatment channel combining water hyacinth ponds and duckweed pond. A pilot waste stabilization ponds channel was operated under the same conditions and simultaneously with the combined channel to allow for comparison. Data analysis was conducted based on average value, standard deviation, variance analysis and principal component analysis.

The overall result showed that the combined WHP/DWP treatment channel is highly efficient under design conditions in treating wastewater and giving an effluent suitable for use in agriculture. Under fluctuating loading rates, the combined channel gave more stable effluent quality than the WSP.

The key conclusions resulting from this study are summarized as follows:

- (1) Anaerobic pre-treatment reduced the amount of suspended solid and organic load. The removal rates were over 65% depending on the influent load. (Chapter 4, Chapter 5 and Chapter 9). In general an increase in the removal rate was observed with an increase in influent load (Chapter 4, 5 and 10).
- (2) The results of the study of WHP operating under three different light intensity conditions (shade, closed room and direct sun light) revealed that the light intensity affects biomass production and pollutant removal in WHP. These ponds performed better under direct sunlight. Ponds under full sunlight will require lower retention time to achieve a same nutrient removal compared to a pond under shade. Under a restricted average daylight intensity of 33 lux, a complete depletion of plants in the pond was observed. The maximum biomass production of 752 t/ha.year and the removal per metre square per day of 711 g, 345 g, 711 g, 731 g and 114 g respectively for COD, BOD<sub>5</sub>, TKN, TN and PO<sub>4</sub><sup>3-</sup> were obtained under full sunlight. The maximum nutrient up-take rates of 6.5 mg/g of FW, 6.5 mg/g of FW and 1.1 mg/g of FW respectively for TKN, TN and PO<sub>4</sub><sup>3-</sup> were observed under full sun.

- (3) WHP operated under different influent pH conditions showed that the pH for optimum treatment and biomass production ranged between 6.4 and 7.1. When the initial pH values moved outside this interval, the plants regulated the pH of the medium to within this range during the treatment process. This adjustment resulted in a drop of the performance of the ponds. However, the observed drop of performance in carbon pollution removal is less pronounced with acidic influent than alkaline influent (Chapter 5). The same adjustment ability was observed with DWP (Chapter 8 and 9).
- (4) The analysis of organic loads removal rate in WHP, over a total retention time of twenty-one days, revealed that more than 80% of the total influent organic load was removed within the first seven days of retention. Therefore there is no need for ponds to have a retention time longer than seven days, which is the optimal retention time for carbon pollution removal. Increasing retention time added very little to efficiency with regard to the organic load removal (Chapter 4, 5 and 7).
- (5) The investigation on Fe, Zn and Cu removal in WHP showed that metal uptake by the biomass of the plants was the main metal removal process in WHP. The removal of the metals studied (Fe, Zn, Cu) by WHP can be described by first order kinetic model with a preferential accumulation sequence of Fe>Zn>Cu. The ponds were effective in the removal of Fe, Zn and Cu with removal rate dependent on the influent solution metal concentration. Removal rates reaching 98%, 96% and 71%, respectively for Fe, Zn and Cu were achieved when the metal content could allow for plant growth. However, toxicity leading to growth retardation was associated with the increase of the concentrations of metals in solution. Moreover, a complete degeneration of the plants was observed when the initial concentration of Cu was 20mg/l. Also, care should be taken in biomass reuse due to the potential risk of the high quantity of toxic elements accumulated in the plant biomass (Chapter 6).

- (6) The accumulation of heavy metals in water hyacinth plants present in pond affected the subsequent treatment processes in ponds. WHP performance and the plant relative growth decreased with an increase in metal contained in the plant bodies. The maximum removal rates of 92%, 88%, 86% and 90%, respectively for TSS, COD, TNK and P-PO<sub>4</sub><sup>3-</sup> were obtained with a control WHP with plants without previous metals accumulation history. These rates dropped to 54%, 53%, 60% and 38%, respectively for TSS, COD, TNK and P-PO<sub>4</sub><sup>3-</sup>, with WHP planted with water hyacinth plants grown in a solution containing a mixture of 10mg/l of Fe, 10mg/l of Cu and 10mg/l of Zn. In addition it was observed that there was a release of the metals in the treated wastewater by the plants, with a higher release rate for the Cu. There is, therefore, a risk of pollution/repollution of effluent by heavy metals (Chapter 7).
- (7) The comparative study of the combined WHP/DWP system and the WSP system revealed that acidic and reducing conditions with low temperature were present in the WHP. In the DWP and WSP, alkaline and oxidising conditions with high water temperature prevailed. However, the pH values for DWP were closer to neutral values and the temperature in DWP were slightly lower than that in WSP. In all the ponds, lower values of pH were observed in the morning and the highest values in the evening. The results of the two systems, tested with ANOVA and visualized with boxplots, show that the gradient of the pH, TDS, e<sub>H</sub> and temperature values was lower for the WHP/DWP channel effluent than that of the WSP channel. The principal component analysis showed that the prevailing temperature depended on the period of the day (Chapter 8). As regards to the performance, WHP/DWP showed better removal of TSS, organic load and nutrient than the WSP. The DO content and the faecal coliform removal were better in the WSP compared to WHP/DWP. The contribution of the WHP to DO and faecal coliform removal was very low and affected the overall removal in the WHP/DWP channel. However these WHP have contributed significantly

to the TSS and organic load removal. The effluents of the two channels were found to comply with the guidelines for reuse (Chapter 9).

- (8) The results on the investigation on the combined WHP/DWP system and the WSP system operated under fluctuating hydraulic and pollutant loading rates confirmed that the effluent quality with regard to nutrient and organic loads was always better in the WHP/DWP than the WSP. The faecal coliform die-off constant fluctuated between 0.36/day and 1.11/day for the WHP/DWP channel and between 0.48/day and 1.88/day for the WSP channel. In general, the faecal coliform removal was better in the WSP than in the WHP/DWP. However at high hydraulic flow rate the removal rate of the WHP/DWP was better even though the effluent from the two systems did not meet always the guideline for reuse. For the WHP/DWP channel, the COD removed was linearly correlated to the influent COD with the same slope for all the loading rates. For the WSP channel, two stages of the linearity existed; a slope of 0.887 was observed at low loading rates and lower slope of 0.218 was obtained for high loading rates. The fluctuation of the loading rates was found to have a significant effect on the WSP channel performance and effluent quality. High variation in effluent quality with regard to TSS, organic load, nutrient and faecal coliform content was observed. In general, the WHP/DWP channel was able to withstand fluctuating influent wastewater quality and hydraulic flow rate, leading to satisfactory performance and more stable effluent water quality than WSP channel (Chapter 9).
- (9) The experiment on fish culturing in the last WSP of the WSP channel and the last DWP of the WHP/DWP channel showed that fish was able to grow in the last DWP. No fish growth was observed in the last WSP.

In general the WHP/DWP is found therefore to be more appropriate to work under fluctuating loading rate than WSP systems.

The research work provided the author with various transferable skills especially in engineering design, process and operational control, environmental management and water quality and integrated resource management. These skills were often shared with numerous visiting researchers and final year master student.

Finally, this study has increased scientific knowledge and improved understanding of the processes governing pollutant removal in water hyacinth and duckweed ponds for optimum system design, and the required conditions for optimum operation and maintenance of macrophyte pond systems for treatment and recycling of wastewater.

## **10.2 Design guidelines and recommendations**

### **10.2.1. Hydraulic retention time**

The hydraulic retention required in macrophyte systems depends on the influent BOD<sub>5</sub>. A total hydraulic retention time (HRT) of between fifteen and twenty-five days is able to reduce the BOD<sub>5</sub> to the required limit depending on the initial load. However, a total retention of over twenty days for the system is advisable for acceptable yield and effluent quality especially as regards to microbial quality. The performance of the system depends on the HTR. The longer the total retention time of the treatment system the higher is the removal. However, a hydraulic retention time of seven day per pond is advisable for optimum removal in each pond.

### **10.2.2. Organic loading rate**

DWP and WHP, as waste stabilisation ponds, are designed based on the organic loading rate expressed as COD or BOD<sub>5</sub>. In a combined WHP/DWP system, the present experiment has proved that the organic removal performance is related to the influent volumetric loading but not on the dissolved oxygen in the medium. The relation between the influent volumetric COD loading ( $\lambda_v$ ) and the removed COD ( $\lambda_r$ ) can be written as follows:

$$\lambda_r = 0.857 \lambda_v + 29.0 \text{ with } R^2 \text{ greater than } 0.98 \quad (10.1).$$

### 10.2.3. Faecal coliform die-off

Maturation ponds are designed, assuming that there is complete mixed conditions in the ponds, by modelling the die-off of faecal coliforms in each pond as a first order kinetic equation (Marais 1974; Metcalf and Eddy 2003). For a series of ponds, the equation is as follows:

$$\frac{N_{if}}{N_{ef}} = (1 + K_T \theta_a)(1 + K_T \theta_1)(1 + K_T \theta_2)(1 + K_T \theta_3)(1 + K_T \theta_4) \quad (10.2)$$

Where  $N_{if}$  is the number of faecal coliforms per 100 ml of the influent wastewater,  $N_{ef}$  is the number of faecal coliforms per 100 ml of the effluent wastewater and  $\theta$ , the retention time (dependant on hydraulic flow rate).

Higher faecal loading than the design loading of the combined macrophyte system is not advisable during system operation due to the risk of having effluent which does not meet the microbial quality set at design.

### 10.2.4. Influent pH, light intensity and metal content

**pH:** The plant biomass production and then the pond performance depends on the influent pH. The experiment showed that the optimum pH for plant growth lined between 6.4 and 7.1. pH outside this range lead to a drop in pond performance; for this reason, when influent water pH is outside this range, for design or prevision of effluent, the drop in removal capacity of the pond should be taken in account.

**Light intensity:** Although there is no specific recommendation on light intensity, the present study showed that water hyacinth growth will decrease when pond is operated under average day light intensity less than 10 000 lux. The growth will cease and a complete depletion will be observed under a restricted average day light intensity less 33 lux.

**Metal content:** The presence of heavy metals in the influent wastewater will reduce the performance of the pond in nutrient and organic load removal rate.

The drop in removal rate depends on the type and the concentration of metal present. Care should be taken when predicting the effluent water quality. Also, due to the risk of complete decay of the water hyacinth plant in pond, WHP cannot be used for the treatment of wastewater containing over 10 mg/l of copper. In addition, when WHP is used for the treatment of wastewater containing a high quantity of metals such as Fe, Zn and Cu, frequent harvesting is required to reduce the risk of repollution of the effluent in metals. Also continue monitoring is necessary to ensure the effluent quality.

#### **10.2.5. Fish culturing**

The combined system is very attractive for fish culturing. However it is suggested that fish should be cultured in ponds with DO always greater than 2 mg/l to avoid mosquito breeding and stress on fish. It is therefore advisable to put fish in DWP and to ensure the DO level is always greater than 2 mg/l before introducing fish.

#### **10.2.6. Construction and protective measures**

In implementing a project of constructing a combined WHP/DWP system prototype in a community, some protective measures should be taken:

- The design of the ponds should provide straight edges and be cleaned frequently to prevent snail breeding and hiding;
- The site should be drained, if possible, to avoid runoff discharge into ponds. Soil obtained from excavation of ponds during construction may be used to form a bund around ponds to prevent runoff entering. It will help to have material to fill in ponds whenever it is decided to close the plant.
- The site should be fenced to protect children from entering the site, animals from drinking the wastewater, especially in dry seasons and to avoid animals falling into ponds.
- The use of the system requires a regular waste water supply.

### **10.2.7. Operation and maintenance**

For optimum result, some measures should be taken during operation and maintenance among which the following:

- It must be ensured that workers wear protective clothing and use disinfectants for cleansing before they go home due to the fact they will be handling sewage and macrophytes directly which may expose them to hazards.
- Periodic harvesting of plants must be adopted. WHP must be harvested on weekly basis and DWP on average twice a week. This measure will prevent DO drop in ponds, also prevent plants from insect attacks and mosquito breeding.
- Other aquatic plants that may grow in ponds have to be removed frequently.

### **10.3 Recommendation for future research**

- 1) The present study has demonstrated the potential of combined WHP/DWP systems to treat and recycle wastewater under fluctuating loading rates. However the effect of parameters such as pH, metal content, light intensity and pollutants loading on the system performance was discussed. Further study is suggested to overcome the need for numerical process modelling, taking into account these different parameters, to predict the effluent quality and resources production ability of such a system.
- 2) The project further revealed that the fish *tilapia* can be grown in last pond of the macrophyte system. The focus of the present work was to demonstrate this ability, but a method to study the growth rate of fish without any harm to them needs to be found. This will allow for cost-effective studying of fish culturing and finding the cost-effective way to optimize fish production rate in the pond. Moreover, better

understanding of the health safety and the nutritional composition of fish produced requires to be checked.

- 3) The results of investigation into the metal removal ability of WHP suggest that high quantities of heavy metal can be accumulated in plant biomass and part may be released into the effluent water; however, the use of effluent sludge and plant biomass harvested as fertilizer or in composting and the use of the effluent water and the fertilizer produced in crop culturing is part of the proposed integrated system. It will be advisable to study the microbial and metal contamination risk of crops and soil due to high concentrations of metal in the influent.
- 4) The research suggested the use of an integrated WHP/DWP macrophyte system at community level to produce income in order to reduce operation and maintenance costs. However the socio-cultural acceptance of crops and fish from the system and the managerial system to be used by the community to ensure that the system will be strictly adhered in order to allow for the sustainability have to be studied.

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