A Sustainable Low-Cost Phytodisinfectant-Sand Filter Alternative for Water Purification

By

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CONTENTS

LIST OF FIGURES IX

LIST OF TABLES IX

DEDICATION X

DECLARATION XI

EXECUTIVE SUMMARY XIII

ACKNOWLEDGMENT XV

LIST OF PUBLICATIONS XVI

EXTENT OF INVOLVEMENT IN PUBLICATION XVII

CHAPTER 1 1

Introduction............................................................................................................1

1.1 Introductory Background..................................................................................1

1.2 Research Questions..........................................................................................3

1.2.1 Poor Water quality for household use in Africa ..................................3

1.2.2 Poor environmental sanitation contaminates drinking water sources....5

1.2.3 Surface water microbiology, poorly documented in rural Africa..........6

1.2.4 Problems and Challenges for Conventional Water Treatment ..........11

1.3 Moringa oleifera Availability and growth requirements.........................12

1.3.1 Origin and Geographical Distribution .................................................12

1.3.2 Classification .........................................................................................12

1.3.3 Vernacular names ..................................................................................13
1.4 Anatomical Description of *Moringa oleifera* ........................................13
1.5 Availability of *M. oleifera* seeds in water treatment .................................14
1.6 Production vis-à-vis International Trade ..................................................15
  1.6.1 Ecology of *Moringa oleifera* ..............................................................16
  1.6.2 Artificial Propagation of *M. oleifera* ..................................................16
  1.6.3 Diseases and pests .............................................................................17
  1.6.4 Conservation Strategies for *Moringa Oleifera* ......................................18
  1.6.5 Breeding ..............................................................................................18
  1.6.6 Genetic Resource base for *Moringa oleifera* .......................................18
1.7 *Moringa* Phytodisinfectant–Sand Filter water treatment ................................20
1.8 Aims/Objectives of the project ..................................................................23
  1.8.1 Significance /Contribution to the discipline ........................................24
  1.8.2 Theoretical framework and Methods ..................................................25
1.9 Indigenous Knowledge, Science and Technology .........................................26

**CHAPTER 2**

28

2.0 Summary ....................................................................................................28

2.1 Introduction/Background ........................................................................29
  2.1.1 Overview of the importance of Water ..................................................30
  2.1.2 Sources of water ................................................................................31
  2.1.3 Microbes in surface water, public health implications ...........................31
  2.1.4 Effects of untreated wastewater on drinking water sources .................32
2.2 A review of Water Treatment in Nigeria and Cameroon ............................34
  2.2.1 Introduction to Water and Wastewater Purification Methods ................35
  2.2.2 Boiling/heating and its setbacks .........................................................38
Chapter 2: Water Treatment Methods

2.2.3 Solar-air Treatment (SODIS) .................................................................................38
2.2.4 The Halogens and their application in water disinfection .........................38
2.2.5 Quaternary Ammonium Compounds and Chlorine Solutions .............39

2.3 Filtration Process .......................................................................................................39
2.3.1 Slow sand filter: Historical evolution .................................................................40
2.3.2 The advantages and defects of the slow sand filter ........................................42
2.3.3 Basic filter Materials and Rationale for use ....................................................43
2.3.4 Candle Filter (porous ceramics), its setbacks ....................................................44

2.4 Coagulants in Domestic Water Treatment ..........................................................44
2.4.1 A survey of Indigenous Plant materials in Water purification ...............45

2.5 The historical use of *Moringa oleifera* plant in water treatment rural Africa .48
2.5.1 Seeds of Moringa Oleifera ..................................................................................49
2.5.2 Botanic Description / Detail Anatomy of *Moringa Oleifera* .....................52
2.5.3 Chemical Constituents of *Moringa oleifera* (lam) ........................................53
2.5.4 *Moringa oleifera* dosing in water treatment .................................................54

2.6 Conclusion and Recommendation .........................................................................56

Chapter 3

Methodology ......................................................................................................................57

3.1 Microbial and Physicochemical analyses of water samples. .......................57
  3.1.1 Plant collection, processing and selection for detail studies .................58

3.2 Processing *Moringa oleifera* plant biomaterials ...........................................59
  3.2.1 Coagulation studies using *Moringa oleifera* powder on turbid water .59

3.3 Construction of Experimental Rigs ....................................................................60

3.4 Preparation of Synthetic Contaminated Water ..............................................62
  3.4.1 Preparation of *Aeromonas hydrophila* for Disinfection studies ........62
3.4.2 Preparation of Synthetic Contaminated water using Soil .................63
3.4.3 Preparation of turbid water using bentonite for Coagulation studies ..63
3.4.4 Preparation of synthetic hybrid turbid and contaminated water .......64
3.4.5 Preparation of model hybrid turbid water with bentonite and soil. .....64
3.4.6 Data collection frequency from the rig experiments. ......................64

3.5 Solvent Extraction of Moringa oleifera (MO) Seeds. .........................65
3.5.1 In-vitro antibacterial assay.....................................................66
3.5.2 Determination of Minimum Inhibitory Concentration (MIC) ..........66
3.5.3 Preliminary Phytochemical analysis of Moringa oleifera extracts.....67
3.5.4 Chromatographic analyses....................................................67

3.6 Construction of a Pilot System ...................................................68

Chapter 4.........................................................................................70

A Moringa oleifera disinfectant sand filter Integration. A review of an alternative
Sustainable technology for household water treatment ......................70

Chapter 5.........................................................................................80

Application of photodisinfectants in water treatment in rural Cameroon ...80

Chapter 6.........................................................................................89

Indeginoous Plant based Coagulants and sand filter media for surface water treatment
in Bamenda, Cameroon......................................................................89

Chapter 7.........................................................................................95

Integrated phytodisinfectant sand filter Drum for household water treatment in
Subsaharan Africa........................................................................95

Chapter 8........................................................................................104

Alternative Perspectives in water and waste water treatment: Phytocoagulant sand
filter alternative for water and water treatment. ..............................104

Chapter 9........................................................................................106

Natural materials for sustainable water pollution management.............106

Chapter 10.......................................................................................140
In vitro Sensitivity of Aeromonas hydrophila to five Polarity based Solvent extracts of *Moringa oleifera*, Alum and Chlorine..........................140

Chapter 11.................................................................158

Phytochemical constituents and comparative antifungal activity of polarity based solvent extracts of *Moringa oleifera* seeds, alum and chlorine on *Aspergillus fumigatus* isolate from Wastewater.......................................158

Chapter 12.................................................................173

An appropriate technology transfer. A sustainable low cost phytodisinfectant- sand filter alternative for water purification...............................173

Chapter 13.................................................................176

Impact and conclusions.................................................176

References........................................................................179

APPENDICES .................................................................192

Appendix 1: Summary of Disinfection of Pathogen Contaminated water pretreated with Moringa extracts before Sand filtration rig (at 45 minutes residence time)The results are submitted for future publication. ...............192

Appendix 2: Coagulation (turbidity removal) levels from Synthetic model turbid water using MO extracts pretreatment and Sand filtration ..........193

Appendix 3: Coagulation and Disinfection of Synthetic turbid water with MO extracts –sand filtration..........................................................194

Appendix 4: Coagulation and disinfection of model synthetic turbid water with MO extracts sand filtration..................................................195

Appendix 5: Treatments with aqueous sequential MO extract sand filtration196

Appendix 6: Treatments with MO salt extract sand filtration ............197

Appendix 7: Treatments with MO crude extract sand filtration.........198

Appendix 8: Treatment with Sand filter (sand filtration) control........199

Appendix 9: Synthetic turbid water with bentonite treated with crude MO seed solution and alum.................................................................200

Appendix 10: Drying Moringa extracts in liquid nitrogen ...............201

Appendix 11: Experimental rigs using for water treatment.........202
Appendix 12: A range of culture media used in the experiments............203

Appendix 13: Pictures of the GTC Pilot MO-Sand Filter system ...........204

Appendix 14: Poster presentation of this research during the Chemical Engineering Conference (CHEMeca, 2010)........................................................205

Appendix 15: Risk Assessment involved in the Research work on A Sustainable Low-cost Phytodisinfectant-Sand filter alternative For Water Purification ........................................................................................................206
LIST OF FIGURES

Figure 1: Children in Bambui, Cameroon, fetching polluted water for household use. 1
Figure 2: Global water supply coverage 5
Figure 3: Sanitation global coverage 5
Figure 4: (a) Moringa seeds and (b) plants 21
Figure 5: Protein structure active coagulant-flocculant ingredient from Moringa seed 22
Figure 6: Particle size distribution curve for Moringa (1) and sand (2) 26
Figure 7: Moringa oleifera seeds and trees, widespread in Sub-Saharan Africa 28
Figure 8: The mechanism of water sedimentation 36
Figure 9: Diagram of coagulation, flocculation and sedimentation 36
Figure 10: Steps of cloth filtration method of treating surface water 40
Figure 11: The formation and function of the biological layer in a slow sand filter 42
Figure 12: Chemical structure of active antimicrobial component in Moringa 50
Figure 13: Moringa seed pod (Yongabi, 2006) 51
Figure 14: Bench scale laboratory rig to demonstrate Moringa-sand filtration 57
Figure 15: Body filter 61
Figure 16: Water filter 61
Figure 17: Location of Cameroon in Africa 68
Figure 18: Community participation and interest on the pilot water project 69
Figure 19: Moringa plant and seed pod (Yongabi et al.,2011) 70
Figure 20: Anti bacterial activity of M. oleifera extracts on E. coli and A. hydrophila 80
Figure 21: Indigenous African plants used to purify water (Carica papaya and Jatropha) 89
Figure 22: Moringa treated water and sand filter setup for household use 95
Figure 23: Back and front covers of a published book emanating from this Research work. 104
Figure 24: First ecological water treatment unit using phyto disinfectant-sand filter system 106
Figure 25: Community mobilization with school children 176
Figure 26: Pilot water filter system (Yongabi et al., 2012) 176

LIST OF TABLES

Table 1: Summary of microorganisms and diseases with Public Health Significance 7
Table 2: Diseases usually related to natural standing or flowing surface water 10
Table 3: Health risks associated with selected water borne pathogens 11
Table 4: Attributes of Moringa peregrina compared with some common Moringa species 19
DEDICATION

To my delightful children:
Othniel David, Chris Francis and Marie Faustina

“The pursuit of truth and beauty is a sphere of activity in which we are permitted to remain children all our lives.”

Albert Einstein
DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any University or Tertiary Institution and, to the best of my knowledge, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. I acknowledge that copyright of published works contained within this thesis (as listed below) resides with the copyright holders of those works:


Kenneth Yongabi, David Lewis Paul Harris (2012). Natural Materials for Sustainable Water Pollution Management. Water Pollution, Prof.Nuray Balkis


Signature: ___________________

Kenneth A. Yongabi

Date: ___________________
EXECUTIVE SUMMARY

In Sub-Saharan Africa, 80-90% of all infectious diseases are water borne. Governments in these countries spend a significant proportion of their budgets importing alum and chlorine from western nations for municipal water treatment. More than 1.2 million people lack safe drinking water in developing countries. Apart from high cost of treating water in sub-Saharan Africa, waterborne microorganisms are developing resistance to currently used disinfectants such as chlorine. To meet the United Nations Millennium Development Goals (MDG) of providing safe drinking water, alternative and complimentary approaches such as the application of *Moringa oleifera* plant materials and sand filters are being studied. Previous research regarding the application of *Moringa oleifera* (MO) seeds have focused on the isolation of bioactive coagulant ingredients for more than four decades, with little attention directed toward field application in small and large scale water treatment applications. Slow sand filters take more than two weeks to generate clean water but there have been few studies directed towards integrating *Moringa oleifera* and other plant disinfectants with sand filters to generate clean water in a relatively short retention times at faster flow rates, generating a more compact filter unit.

This research sought to fill this knowledge gap. Quantitative research techniques were applied to test a Moringa-sand filter column for its disinfection activity on separate synthetic contaminated water containing *E. coli*, *Aeromonas hydrophila*, total heterotrophic soil bacteria and fungi. The constructed Moringa-sand filter column was analyzed for its coagulant activity using synthetic turbid water made from bentonite and soil. Further research into documentation of indigenous knowledge and the use of indigenous medicinal plants in Cameroon with a history of use in purifying water was carried out at both the Phytobiotechnology Research Laboratories in Bamenda, Cameroon and the Microalgae Research Laboratory of the School of Chemical Engineering, Adelaide University. The coagulant and disinfection ability of the plants using surface contaminated water was carried out at the Phytobiotechnology Research Laboratories in Cameroon, followed by in-vitro antimicrobial activity of the organic extracts using microbial isolates from stream water in Bamenda, Cameroon. The coagulant and disinfection potential of *Moringa oleifera* seed extracts were superior to other plant materials. To this effect, further studies on *Moringa oleifera* seeds were planned and executed at the Microalgae Laboratory, School of Chemical Engineering, Adelaide University. Extracts of *Moringa* seed powder using solvents of varying polarity revealed more than 85% in-vitro antibacterial activity against *E. coli*.
(ATCC11775) strain (indicator of faecal contamination of water) and 95% against *Aeromonas hydrophila* strain (known to resist chlorination) compared to control of both organisms of 65% for aluminium sulphate and 80% for sodium hypochlorite. Phytochemical screening and chromatographic analyses were carried out to elucidate the possible bioactive disinfectant ingredient in *Moringa* seeds. These experiments were conducted as proof of concept and were preceded by an evaluation of the microbial content of surface water at Bambui and Mile 6 Mankon water sources used for household chores in Bamenda, Cameroon, for total bacterial counts, *E. coli* and coliform counts. A pilot low cost disinfectant sand filter system was set up at the Government Technical College, Njinikom, in Cameroon; to test its disinfectant and coagulant efficiency using total bacterial count, *E. coli*, coliform and fungal counts, pH, turbidity and to provide a capacity building on dissemination of this knowledge at household level in Cameroon.

Data was collected every 24 hours for a period of a month for the bench experiments using sand filters and for field work for 12 months on the pilot plant. The mean bacterial counts, pH, turbidity and a catalogue of plant materials used in water treatment were recorded.

The main findings of this research are presented as a series of six publications consisting of four peer-reviewed journal articles, a book, a book chapter as well as two manuscripts submitted for publication:

The main findings of this research were applied in a pilot water project at Government Technical School Njinikom, Boyo division, Cameroon. It was found in the pilot study that *Moringa* pretreated water filtered through sand media met both the Australian and the World Health Organization guidelines for drinking water. The broad lessons for water purification are that the use of locally available natural coagulants and disinfectants in resource limited countries has a great potential of improving the economy and health of the people. This research has demonstrated the efficacy of both plant based coagulants, disinfectants and sand filter media through extraction, in vitro bioassay, purification and integration of the two systems. It is highly recommended that governments in poor countries should take up this technology. It will require commitment of countries to strengthen the natural water coagulation technology in a holistic, integrated approach and to support initiatives including empowering and enabling local scientists to build up this system at the grassroots level.
ACKNOWLEDGMENT

A number of people participated in this research. Most importantly, I thank the principal, staff and students of the Government technical college, Njinikom, Cameroon, who worked relentlessly toward the construction of the *Moringa* pilot water treatment plant. I remain grateful to the technical staff of the School of Chemical Engineering, The University of Adelaide, who were very supportive towards the design and fabrication of the experimental Moringa-sand filter rigs. In particular, I owe thanks to Jason, Jeff, Mike and Andrew Wright. My greatest thanks are reserved for Ass.Prof. David Lewis and Mr. Paul Harris, who accepted supervision of me. Their persistent support and enduring patience provided an extraordinary inspiration and made this work a truly exceptional learning curve for me. I enjoyed David and Paul’s wonderful blend of social, industrial and academic experience. To Dr. William Donohue for his academic and social support throughout my stay at Adelaide.

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To Jess, Brendan and Frances (above L-R), with whom I worked closely on this project. They have been very inspirational with their thoughtful questions. Their interests in sustainable engineering are unequivocal. We were privileged to have one of our Honors students on the water quality testing component of this project, Brendan Moore, receive the prize for best poster and Honors Thesis presentation.

To Dianne Parish for her interest in this work and who encouraged Brendan, Jess and Frances to contact me. To Dana Thomsen, the Harris’ family and the good people of Gawler Baptist Church for their moral support throughout my stay at Adelaide.

The entire students, Administrative and academic staff of the school of Chemical Engineering were very supportive throughout my stay in Adelaide and I thank them all.

To God, is all the glory for the gift of life.
LIST OF PUBLICATIONS

The following publications have arisen from the research conducted during the study period and are included in the thesis as individual chapters, contributions of co-authors are described in authorship statements that appear prior to each article.

Chapter 4 – Article 1

Chapter 5 – Article 2

Chapter 6 – Article 3

Chapter 7 – Article 4

Chapter 8 – Article 5

Chapter 9 – Article 6

Chapter 10 – Article 7

Chapter 11 – Article 8


Additionally, a poster on “Cheap biocoagulant sand filter system for water purification in low income earning countries” was presented at Chemical Engineering conference (CHEMECa), 2010 in Adelaide (Appendix 14)

**EXTENT OF INVOLVEMENT IN PUBLICATION**

The bench scale laboratory work was done in the microalgae laboratory, School of Chemical Engineering, The University of Adelaide. The survey of indigenous biocoagulants and laboratory screening for antimicrobial activity was carried out at the Phytobiotechnology Research Laboratories, Bamenda, Cameroon.

All the peer-reviewed articles, book and book chapter are multi-authored but I am the lead author on each. Descriptions of the involvement of each author and their agreement to the inclusion of the manuscript in this thesis are provided in the authorship statement at the start of each chapter in which each manuscript is reproduced in this thesis. A brief overview of the involvement of the authors in each article is provided below.

Article 1: This was carried out by me and is based on my research findings with advice and editorial assistance from Dr. David Lewis and Paul Harris.

Article 2: Drawn from the research work I did in Cameroon and Adelaide. I wrote the article and Dr. David Lewis and Paul Harris went through the draft and provided editorial comments.

Article 3: I drafted this article, and both Dr. David Lewis and Paul Harris edited the draft.

xvii
Article 4: I drafted this article; Dr. David Lewis provided a critique. The final draft was re-edited by Dr. David Lewis and Paul Harris and the quality greatly improved.

Article 5: I prepared and submitted the draft of this article. Significant modifications to the Manuscript were required as a result of the reviewers’ comments, and I undertook the process of revising the manuscript. Dr. David Lewis and Paul Harris approved the final product.

Article 6: This chapter was drafted by me and Dr. David Lewis and Paul Harris went through and approved its publication.

Article 7: I prepared the manuscript and Drs. David Lewis and William Donohue made editorial comments. I revised the manuscript before submission.

Article 8: I prepared the manuscript and Dr. David Lewis went through for editing. His comments were incorporated into the work before submission.

All the chapters of this work are the result of my research work with editorial advice from Dr. David Lewis and Paul Harris

Kenneth Yongabi.

October, 2012
CHAPTER 1

INTRODUCTION

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Figure 1: Children in Bambui, Cameroon, fetching polluted water for household use.

This research project is focused on investigating the efficacy of a sustainable phytodisinfectant sand filter alternative for water purification. Chapter one generally provides background knowledge on the water crisis, water borne diseases and the importance of developing sustainable water treatment technologies. The fundamental knowledge of this research is more likely a general outline of the short falls of the current water treatment technologies and the potential benefits of integrating a *Moringa oleifera* seeds onto a sand filter system. A comprehensive up to date literature review was carried out in the course of this study.

1.1 INTRODUCTORY BACKGROUND

Approximately five million lives are lost annually due to drinking and use of contaminated water (Madore *et al*., 1987; WHO, 2006; Pritchard *et al*., 2009 and Amir *et al*., 2010). Globally, four billion cases of diarrhea causing one point eight million deaths are reported every year (Madsen *et al*., 1987). Out of this, 90% are children below the age of five (UNESCO, 2007 and Pritchard *et al*., 2009). In sub Saharan Africa, diarrhea morbidity is about 17% (Masangwi *et al*., 2008). Water scarcity and poor environmental sanitation is increasing. This is mainly due to the disproportionate increase in population, global climatic change, drought cycles, deforestation and desertification, particularly in Sub Saharan Africa (Muyibi, 2001). The population of Sub Saharan Africa is increasing steadily at an
unprecedented rate of 2.6% per annum (Adegbola, 1987). In Nigeria, for instance, population increases have been approximated at 3.0%, while in Ghana and Burkina Faso, 2.9% (Anon, 1998), the trend is similar for many countries in Sub-Saharan Africa. This implies increasing pressure on water resources (Schultz and Okun, 1983).

Ground and surface water is the main source of drinking water for more than 70% of the population in Africa (Muyibi and Alfugara, 2003; Muyibi et al., 2003; Pritchard et al., 2009). The most common sources of drinking water in Africa, particularly for Cameroonians, are streams, boreholes, shallow wells, springs and rivers (McConnachie et al., 1999 and Muyibi et al., 2002a). These water sources are unprotected (Staines, 2002) and usually consumed without any form of treatment (Pritchard et al., 2009). A number of waterborne and water related diseases such as typhoid, paratyphoid, diarrhea, cholera and dracunculosis respectively are fast becoming endemic in most developing countries (Godfrey, 2003 and Mingquan et al., 2006). These pathogens are commonly derived from human faecal material (Schultz and Okun., 1984; Pritchard et al., 2007; Pritchard et al., 2008). In the rainy season, many pit latrines in developing countries collapse under their own weight due to poor construction, further reducing sanitation coverage. Furthermore, open defecation in the bushland and in water bodies is still a common means for human excreta disposal for rural populace without access to pit latrines (Pritchard et al., 2009). In the rainy season, faecal matter from pit latrines and open sources is washed into surface water thereby contaminating the water (Dzwairo et al., 2006). In Malawi, for example, microbiological water quality from shallow wells (with depths typically not exceeding 15 m) has been found to be more inferior in the wet season compared to the dry season (Pritchard, 2007; Pritchard, 2008 and Pritchard et al., 2009). However, the situation could have easily been eradicated through proper sanitation and provision of adequate clean water (Yongabi, 2004 and Pritchard et al., 2009). In developing countries, particularly in Africa, water treatment is quite expensive, the ability to pay for services is very low and low cost technology scarce. In Cameroon and Nigeria, for example, aluminum sulphate is imported from Europe at a cost of 60USD per kilogram and calcium chlorite at 45USD per litre. In most African countries more than 70% of its populace live below the poverty line (Crapper et al., 1973; Christopher et al., 1995; Kagwa, 2001; and GOM, 2005). Reports for more than three decades suggest strongly that this trend could be reversed if focus is laid on sustainable water treatment systems that are low cost, simple and requiring minimal maintenance (Olsen, 1978; Jahn, 1979; Schultz and Okun, 1984; Jahn, 1984; Jahn, 1986; Madsen et al., 1987; Muyibi and Okuofu, 1995; Pollard et al., 1995; Muyibi et al.,
Generally, standard water treatment involves a number of unit processes depending on the quality of water source in question (Nalm et al., 1998), cost and existing guidelines (Kebreab et al., 2005) and no one method is devoid of pitfalls (APHA, 1983 and AWWA, 1995).

There are some water bodies that are naturally defective due to the geology of the area. For instance, an area with a lot of limestone would produce hard water (Muyibi, 2001). In addition, there are also natural and artificial water bodies like ponds that contain a lot of nutrients and are unacceptable for consumption. This implies that an appropriate treatment would have to be applied (Jahn, 1986 and Jahn, 1988).

It has been estimated that 125 liters of water (potable) is required per person per day (UNICEF, 2009). However, many households are unable to get 25 liters of clean water per person per day due the high level of contamination. Water purification technologies would have to be reviewed in terms of its simplicity, accessibility (cost) and efficiency. Conventional methods of assuring potable water in developing countries are unsustainable so there is a need to consider the application of sustainable technologies using locally available materials in treating surface water (Pritchard et al., 2009).

One sector whose secrets hold a lot of promise for the future is the plant kingdom (Dorries, 2005; Dalen et al., 2009). There is a rich bio-diversity of plants that could be explored for sustainable low cost water treatment (Diaz et al., 1999). The search for a simple, reliable and effective method of water treatment has led to the use of plant materials including seeds of Moringa oleifera (Eilert, 1978; Fuglie, 1999, Folkard et al., 2000 and Yongabi, 2004). The integration of a locally available plant disinfectant and coagulant - Moringa oleifera - with sand filtered water could provide a sustainable alternative water treatment.

1.2 **Research Questions**

1.2.1 Poor Water quality for household use in Africa

Potable water supply at an affordable cost is a major problem in most parts of Africa that needs to be addressed (Masangui et al., 2008). In urban areas, where conventional water is treated using synthetic coagulants and disinfectants, the quality of the water is often compromised by improper use of chemicals (Dada et al., 1990 and Dalen et al., 2009). For example, rota virus in tap water treated with synthetic coagulants and disinfectants has been
reported in Ghana (Julius et al., 2010). In Cameroon reports in Yaoundé, the capital city, indicate that municipal water contain worms (Peter, 2007). Contamination during storage of treated water and during distribution has been observed (Godfrey, 2003; Lilliehook, 2005 and Masangui et al., 2008). This continues to compromise water quality below the WHO standards (WHO, 1998; WHO, 2006). There is a high level of turbidity (greater than 400NTU) and coliform counts greater than 10 colonies per ml of surface water, often used for household chores (Josephson et al., 1997 and Pritchard, 2008). This has been partly caused by runoff during the wet season. Increased population leading to crops being cultivated in areas closer to the banks of rivers, which are reservoirs of water supplies to the cities, is another factor. As a result of this, the soil in this area loosens and is more prone to removal by runoff, thus creating a turbidity problem all year round (Kaggwa et al., 2001; Lilliehook, 2005 and Pritchard et al., 2009). This adds to cost of treatment (Kebreab, 2005). The lack of suitable technology (Jahn, 1986), limited skilled work force (Sutherland et al., 2000) and insufficient funds have been identified as part of the problem. Besides, household contamination of water stored in buckets is a major problem in Africa (Godfrey, 2003).

In Nigeria, for instance, a local survey carried out in the Bauchi, Plateau and Benue states shows that potable water is unavailable for more than 70% of the rural inhabitants (Yongabi, 2004). Furthermore, many villagers walk for more than 5 kilometers to fetch dirty water in polluted streams for domestic use. Fig 1 below shows the map of the world indicating global water supply coverage. The areas marked in red, brown and purple indicate poor water supply coverage and more than 85% of Africa has poor water supply. In Fig.2, the red, brown and purple marks indicate poor sanitation coverage. More than 85% of Africa, Middle East and parts of South America have poor sanitation coverage.

It is believed that a household Moringa–sand filter system developed in this research could treat any form of turbid water within a relatively short residence time of an hour and with faster flow rates.
Chapter 1

Red indicates poor water supply (0-25%); Brown indicates (25-50% coverage); Yellow indicates (51-75% coverage); Green (76-90% coverage); Blue indicates (91-100% coverage) and White indicates Missing data

Figure 2: Global water supply coverage

Red indicates poor sanitation coverage (0-25%); Brown indicates (25-50% coverage); Yellow indicates (51-75% coverage); Green (76-90% coverage); Blue indicates (91-100% coverage) and White indicates Missing data

Figure 3: Sanitation global coverage

1.2.2 Poor environmental sanitation contaminates drinking water sources
Disposal of animal, human and industrial wastes constitutes one of the major sanitation problems in rural and semi-urban Africa (Khan, 1984; Yongabi, 2009). Animal waste, traditionally sprayed on the fields as fertilizers, is a potential hazard to grazing animals and contaminates underground and surface water resources, which are fetched for drinking and other potable uses (Dzwairo et al., 2006 and Dungumaro, 2007). Cattle and poultry manures play host to a number of bacterial, viral, fungal and parasitic diseases such as salmonellosis, taeniasis, cryptosporidiosis, giardiaris, entamoebiasis, campylobacteriosis, listeriosis, aspergillosis, candidaisis, rota viral disease and hepatitis (Cheesbrough, 1984; Lisle and Rose, 1995; Lechevallier and Norton, 1995).

Untreated wastewater from several sources, including food processing factories, carry infective stages of these microorganisms (Bove et al., 2002). This is disposed of untreated (Staines, 2002) and potentially contaminates underground water sources used for drinking (Cheesbrough, 1984 and Jahn, 1986). The faeces of cattle as well as wastewater from meat processing plants are a vehicle for a diverse range of microorganisms. In most African communities, the faeces of cattle are used as organic fertilizers. This potentially leaks and contaminates drinking water sources (Jones, 1980; Larsen and Munch, 1982 Lilliehook, 2005). The major cities in Africa are rapidly experiencing over-population as a result of industrial growth, particularly small-scale agro-based enterprises that utilize water are steadily increasing. The implication of this is an ever growing increase in the domestic and industrial effluents resulting in increased surface water contamination. It is hypothesized that the local plant coagulants identified and screened in this study, as well as the Moringa disinfectant-sand filter system, can be used by industries to treat turbid water at low cost thereby improving water quality.

1.2.3 Surface water microbiology, poorly documented in rural Africa

The detailed microbiology of surface water is an important step toward disinfection (Cheesbrough, 1984; Broin et al., 2002). However, the microbiology of most drinking water sources in most parts of rural communities in Africa is poorly documented (Ndabigengesere et al., 1995; Ndabigengesere and Narasiah, 1998; Yongabi, 2004; Amir et al., 2010) and also in other developing countries (Ozacar and Sengil, 2002). This is because most studies rely on the traditional indicator organisms such as faecal coliform and total coliforms to corroborate water microbial contamination (Efstratious et al., 1998). Recent studies suggest that microbes in water are so diverse (Tables 1, 2 and 3) that some species may not correlate well with indicator organisms (Landre et al., 1998). Certain species are viable but may not grow on bacteriological media (Landre et al., 1998), so the dependence on indicator organisms alone may not be adequate when testing the efficiency of water purification
Chapter 1

The rate of contamination of surface water in most African countries is constant (Lilliehook, 2005). Waste water is being disposed of in river banks; river silt is churned into suspension and runoff from fields during the wet season. This implies that constant monitoring of water quality and appropriate treatment is necessary (Fujikawa et al., 1997; Sutherland, 1994). Tables 1, 2 and 3 show a list of water borne and water related diseases and their public health significance and health risks. In this research, a field analysis of the microbiology of surface water in Bambui and Mile Six Mankon in Cameroon was carried out; indicator organisms, specific isolation details as well as their counts was undertaken to validate the efficiency of the disinfectant-sand filter system.

Table 1: Summary of microorganisms and diseases with Public Health Significance

<table>
<thead>
<tr>
<th>Group of disease</th>
<th>Disease and Pathogen</th>
<th>Transmission route</th>
<th>Related measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water acts as a passive vehicle for the infecting agent.</td>
<td>Cholera (vibrio cholera)</td>
<td>I</td>
<td>Improve-water quality</td>
</tr>
<tr>
<td></td>
<td>Typhoid (salmonella typhi)</td>
<td>I</td>
<td>aim for maximum</td>
</tr>
<tr>
<td></td>
<td>Leptospirosis Giardiasis (Giardialamblia )</td>
<td>P,C</td>
<td>microbiological quality of water improve</td>
</tr>
<tr>
<td></td>
<td>Amoebiasis (Entamoeba histolytica)</td>
<td>O</td>
<td>sanitation</td>
</tr>
<tr>
<td>Water-washed Diseases</td>
<td>Scabies</td>
<td>O</td>
<td>Improved quality of water (adding chloride,</td>
</tr>
<tr>
<td></td>
<td>Skin sepsis</td>
<td>C</td>
<td>water (adding chloride,</td>
</tr>
<tr>
<td></td>
<td>Yaws</td>
<td>C</td>
<td>alum and lime) provide a</td>
</tr>
<tr>
<td></td>
<td>Leprosy</td>
<td>C</td>
<td>greater volume of water,</td>
</tr>
<tr>
<td></td>
<td>Lice and typhus</td>
<td>C</td>
<td>facilitate access and</td>
</tr>
<tr>
<td></td>
<td>Trachoma</td>
<td>C</td>
<td>encourage its use -</td>
</tr>
<tr>
<td></td>
<td>Conjunctivitis</td>
<td>C</td>
<td>improve sanitation and</td>
</tr>
<tr>
<td></td>
<td>Bacillary dysentery</td>
<td>B</td>
<td>personal hygiene</td>
</tr>
<tr>
<td>Intestinal infections in this group as due to poor human waste disposal</td>
<td>Shigella sonnei</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Escherichia coli)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salmonelosis</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Salmonella typhimurium)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Paratyphoid fever</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salmonella paratyphi</td>
<td>O</td>
<td></td>
</tr>
</tbody>
</table>
Chapter 1

Table 1 Continued

<table>
<thead>
<tr>
<th>Group of disease</th>
<th>Disease and Pathogen</th>
<th>Transmission route</th>
<th>Related measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water based</td>
<td>Virus diarrhea (Rota virus)</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>disease</td>
<td>Ascariasis (Ascaris lumbricoides)</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>transmitted</td>
<td>Whipworm (Enterobiasis)</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>through aquatic</td>
<td>Enterobius vermicularis</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td>invertebrates.</td>
<td>Urinary Shistosomiasis (Schistosoma</td>
<td>P</td>
<td>Reduced contact with infected water.</td>
</tr>
<tr>
<td></td>
<td>haematobium)</td>
<td></td>
<td>Protect water source</td>
</tr>
<tr>
<td></td>
<td>Rectal Shistosomiasis (Schistosoma</td>
<td>P</td>
<td>Improve sanitation</td>
</tr>
<tr>
<td></td>
<td>mansoni)</td>
<td></td>
<td>(Chlorinating the effluent)</td>
</tr>
<tr>
<td></td>
<td>Dracunculosis (guinea-worm) (Dracunculus</td>
<td>O</td>
<td></td>
</tr>
<tr>
<td></td>
<td>medinensis)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A necessary part of the life cycle of the infecting agent takes place in an aquatic animal. Some are also affected by waste disposal. Infection spread other than by contact with or ingestion of water have been excluded.

Water based disease transmitted through aquatic invertebrates.
Water-related vectors.

Infections are spread by insects that breed in water or bite near adequate pope supplies with water storage jars where the insects breed unaffected by waste disposal. Onchocerciasis is acquired only by eating uncooked fish or other large aquatic organisms.

<table>
<thead>
<tr>
<th>Group of disease</th>
<th>Disease and Pathogen</th>
<th>Transmission route</th>
<th>Related measures</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water-related vectors</strong></td>
<td><strong>Yellow fever (Yellow fever virus)</strong></td>
<td><strong>B (mosquito)</strong></td>
<td></td>
</tr>
<tr>
<td>Infections are</td>
<td>Dengue dengue hemorrhagic fever</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>spread by insects</td>
<td>West/Nile + Rift valley fever</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>that breed in</td>
<td>Arbovirus</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>water or bite near</td>
<td>Malaria</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>it adequate pope</td>
<td>Plasmodium (Plasmodium falciparum)</td>
<td>B</td>
<td></td>
</tr>
<tr>
<td>supplies with water</td>
<td>Onchocerciasis (onchocerca vulvulus)</td>
<td>B (simulium fly)</td>
<td></td>
</tr>
<tr>
<td>storage jars where the insects breed unaffected by waste disposal</td>
<td>Sleeping sickness (Trypanosomiasis)</td>
<td>B (Tsetse fly)</td>
<td></td>
</tr>
<tr>
<td>Trypanosoma rhodiense</td>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leishmaniasis (Leishmania donovani)</td>
<td>B</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Faecal Disposal disease.** A group of water base type infection to be acquired only by eating uncooked fish or other large aquatic organisms.

- **Fish** from contaminated water: Proper disposal of fecal material
- **Fish** from contaminated water: Eat well cooked fish
- **F** for Fish
- **U** for Urine
- **P** for Percutaneous
- **C** for Cutaneous

Key:

- F → Fish
- O → Oral
- N → Nose
### Table 2: Diseases usually related to natural standing or flowing surface water

<table>
<thead>
<tr>
<th>Group of diseases</th>
<th>Disease and pathogen</th>
<th>Route entering man (a)</th>
<th>Route leaving man (b)</th>
<th>Related measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>(b) Food borne disease acquired through ingestion of the organisms in food (contaminated food) improperly preserved food</td>
<td>Salmonellosis salmonella typhimurium, S. enteritidis Bacillary dysentery Campylobacter jejuni Campylobacter coli E. coli Shigella sonnei Entero virus Entamoeba histolytica Giadia lamblia Aspergillosis (Aspergillus fumigatus fungus)</td>
<td>Through contaminated food and water, ingestion of cysts, larva on vegetables</td>
<td>Improper disposal of faces, good water quality, supply and sanitation practice washing of hands before meals Avoid using livestock wastes as fertilizers.</td>
<td>Proper disposal of faces, good water quality, supply and sanitation practice washing of hands before meals Avoid using livestock wastes as fertilizers.</td>
</tr>
</tbody>
</table>
1.2.4 Problems and Challenges for Conventional Water Treatment

In many developing countries, proprietary chemical coagulants such as aluminum sulphate and other synthetic polyelectrolytes are either unavailable (Zeng or Park, 2009) or available at a high cost (Boisvert et al., 1997; Kebreab et al., 2005). In Malawi, for instance, the government spent more than 25000 pounds in 1998 and more each year on the purchase of aluminum sulphate for water treatment (Pritchard, 2007). Similarly, in Nigeria, some municipalities are spending around 50% of their annual recurrent budget on water supplies (Muyibi, 1995). Other conventional technologies such as distillation, ion-exchange, (deionization), carbon adsorption, ultra filtration, reverse osmosis and ultraviolet radiation are not widely available in most parts of Africa as they are much more expensive than the use of alum and chlorine (Muyibi, 1995). These advanced technologies also depend on high energy input, for which most rural areas in Africa do not have sufficient electricity (Nkhata, 2001).

Tap points in most African cities are usually crowded with long queues because of slow water flow rates (Dungumaro, 2007). This has generated lot of social ills. Furthermore, reports suggest that household water recontamination poses a grave danger to

---

### Table 3: Health risks associated with selected water borne pathogens

<table>
<thead>
<tr>
<th>Health risk</th>
<th>Type of pathogen</th>
<th>Found in</th>
<th>Waterborne</th>
<th>Foodborne</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute parotitis</td>
<td><em>Anaerobacteriaceae</em></td>
<td>-</td>
<td>+ (recreational)</td>
<td>+</td>
</tr>
<tr>
<td>Antritis</td>
<td><em>Giardia, Salmonella, Campylobacter</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aspergillus meningitis</td>
<td><em>Echovirus, Coxsackie virus</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cancer, postic ulcer</td>
<td><em>Helicobacter pylori</em></td>
<td>H/− (presumed, in fecal)</td>
<td>H/− (preliminary in groundwater)</td>
<td>+</td>
</tr>
<tr>
<td>Cholera</td>
<td><em>V. cholerae</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Diarrhea and gastroenteritis</td>
<td><em>Many enteric viruses</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>Norwalk virus</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>Giardia, Cryptosporidium</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Salmonella, E. coli O157</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>Shigella species, Salmonella</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>Caldwuris</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>Rotavirus (infantile gastroenteritis virus)</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>Astroviruses, enteroviruses</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>Mycobacterium avium</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Heart disease</td>
<td><em>Coxsackie B virus</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Insulin-dependent diabetes</td>
<td><em>Coxsackie B virus (159)</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Kidney failure</td>
<td><em>E. coli O157/H1</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>Microsporidia</em></td>
<td>+</td>
<td>+</td>
<td>+/−</td>
</tr>
<tr>
<td></td>
<td><em>Cyclospora</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>V. vulnificus</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Liver failure</td>
<td><em>Hepatitis A virus</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>Hepatitis B virus</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>Hepatitis E virus</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>V. angewiticus</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Wound infection</td>
<td><em>V. parahaemolyticus</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*+ refers to being associated with; −, not associated with; H/−, possible or suspected in linkage*.
health (Godfrey, 2003). Recently, there are disturbing reports of chlorine resistant organisms such as, cryptosporidium oocysts, strains of salmonella sp, entamoeba cyst, mycobacterium sp, Escherichia coli 0157:47 and particularly aeromonas hydrophila (Pritchard et al., 2009). Additionally, rota virus has been detected in treated tap water supplies in Ghana (Julius et al., 2010). This further suggests that these organisms may have developed tolerance to chlorination over time (Julius et al., 2010).

There is a need to explore, exploit and enhance indigenous knowledge by the application of low-cost, simple and effective technologies using plant based disinfectants integrated onto a sand filter system.

1.3 Moringa oleifera Availability and Growth Requirements

An overview on the species Moringa oleifera is presented. The attributes of this species based on: Origin and geographical distribution, classification, common names, anatomy and applications of tree products with a focus on water purification. International trade, aspects of ecology and artificial propagation, diseases and biodiversity issues amongst others are also highlighted. Moringa oleifera is widely distributed and better known for its coagulant properties than any of the fourteen species in the Moringa family. Moringa oleifera as a coagulant is more available in Sub-Saharan Africa than Aluminum sulphate and chlorine. Approximately, 1.6 hectares of Moringa oleifera (600,000 seeds) is required to treat one million litres of turbid water a day. The seed of Moringa peregrina is much oilier than the well-known species Moringa oleifera. Proximate analyses of the M. peregrina oils possess 20-50% oil with high culinary, cosmetic, industrial and therapeutic potentials. Moreover, all the plant biomass has a wide range of applications.

1.3.1 Origin and Geographical Distribution

Moringa oleifera (MO) occurs naturally in arid or semi-arid countries spanning from Western Somalia to Israel. MO was first identified in India, and specifically reported in Western Somalia, Sudan, lowland areas around the Dead and Red seas, Egypt, Yemen, Saudi Arabia (Arabian Peninsula), United Arab Emirates, Syria, Palestine and Israel.

1.3.2 Classification

It belongs to the species; Moringa oleifera by Lam (1795) and a protologue flora Aegyptia co-arabica as reported by Forsskal (1775). Moringa oleifera, like other Moringa species, is a member of the family Moringaceae, order Brassicales, in the class of magnoliopsida in the division magnoliophyta.

Generally, this division comprises of one genus and thirteen species. The other species of Moringa are; Moringa arborea, Moringa borziana, Moringa concanensis, Moringa
drouhardii, Moringa hildebrandtii, Moringa longituba, Moringa oleifera, Moringa ovalifolia, Moringa pygmaea, Moringa rivae, Moringa ruspoliana and Moringa stenopetala.

*Moringa oleifera*, like many other species in the family, has been variously called *Moringa pterygospermae* Gaertn (1791); *Hyperanthera peregrine* Forssk (1775) for *Moringa peregrina*, *Gymnocladus arabica* Lam (1785); *Hyperanthera semidecandra* Vahl (1790); *Hyperanthera arborea* Gmet. (1791); and *Moringa Arabica* Anon. (1805). Amongst these, the synonym *Moringaaptera* Gaertn (1791) has been reported as the closest.

1.3.3 Vernacular names

*Moringa oleifera* Lam is commonly called in English Ben tree; wispy-needled yasar trees and Murunggi in Malayan. The seeds are called ben nuts while the resulting oil from the seed is called oil of ben. Other names are; wild drum stick and rocky wash, names that indicate the kind of habitat in which the tree grows.

In Sudan, the name “Shagarat al aruwaq” is an unspecific name for all the *Moringa* species which means- the plant that purifies water - this probably holds true for middle belt and northern region of Nigeria where the appellation “Jegede” and “Zogale” respectively is generally used for the *Moringa* species (Yongabi, 2004) while in Oman, it is specifically called “Shuh”.

1.4 ANATOMICAL DESCRIPTION OF *MORINGA OLEIFERA*

Amongst the Moringaceae, *M. oleifera* is the smallest species in terms of its morphological relationship with other *Moringa* species. For instance, *Moringa oleifera* lam more closely resembles *M. concanensis* than *M. peregrina*, while *M. drouhardii*, *M. hildebrandtii*, *M. ovalifolia* and *M. stenopetala* show a very close resemblance anatomically and are called “bottle trees”.

The seedling of *M. oleifera* is tuberous and has broad leaflets. As it grows, the leaves get smaller and widely spaced before they are eventually transformed into needle-like leaves that drop at maturity. The overall architecture of the mature tree closely resembles that of tamarind trees.

Before it becomes a permanent tree, *M. oleifera* undergoes a series of diebacks becoming slender. The process of die back is not unique to this plant but also occurs in other plants such as *Pterocarpus angolensis*, a characteristic of trees growing in areas of limited rainfall, high temperature and/or those areas regularly devastated by wild fires. It is an
evergreen species with needle–like leaves. The flower morphology of *Moringa* species has been studied extensively. The works of Orson (2003) described it as having five sepals, five petals, five antesepalous staminodes, and five antepetalous stamens with monotheral and bisporangiate anthers.

The ovary is borne on a gynophore of varying length, with a hollow style. In addition, there are nine zygomorphic flowered species that constitute a monophyletic group (the “zygomorphic clade”). Zygomorphy in the flower is a common feature among all the species, as a result of the differential flexion of petals, sepals, the androecium, and the gynoecium. Cream-colored flowers have been observed in the Asian species of *Moringa*, but are also widespread in Africa.

*M. oleifera* has bluish pinnate leaves with naked leaf axes. During flowering and fruiting periods, it displays its splendid pink flowers and its long angular pods. The pink/white zygomorphic flowers of *M. oleifera* are sweetly scented and thus attract a lot of foraging bees. Apart from dropping the leaflet at maturity, the pod split lengthwise thus releasing and dispersing pea-size seeds.

The wood anatomy of *M. oleifera* such that growth ring boundaries are either not distinct or absent in some, wood is diffuse and porous with simple perforation plates. The inter vessel pits alternate – shape of alternate pits is polygonal and measuring 7 – 10 micrometers and can be greater than 10 micrometers. The vessel-ray pits possess much-reduced borders to apparently simple but vary from round, angular, horizontal (scalari form, gash-like) to vertical (palisade) and with an approximate dimension of 100 – 200 micrometers. There are 5 to 20 vessels per square millimeter with diameter ranging from 350-800 micrometers. Furthermore, tyloses are common, with fibers appearing simple with bordered pits. Non-septate fibers are also present. The structures of the fibers, which have been extensively studied, reveal that they vary from thin to thick walled with a dimension of 900 micrometers. The axial parenchyma has a number of shapes; from vasicentric, aliform, confluent to fusiform. Two to four cells per parenchyma strand have been observed. The ray width contains between one to three cells. Ray cells are procumbent with one row of upright and or square marginal cells of diameter between 4 – 12 mm. Generally, all rays and fibers are storied but some rays and or axial elements are irregularly storied. Prismatic crystals are also present in procumbent ray cells while some are present in non-chambered axial parenchyma cells. Equally, there are others mostly smaller crystals of various shapes that have been observed.

### 1.5 Availability of *M. oleifera* Seeds in Water Treatment
A number of uses and applications of *M. oleifera* notably, its oil and coagulant potential has been identified (Polyprasid, 1993 and Tsaknis, 1998). The oils have a high culinary, cosmetic, pharmaceutical and industrial value. The seeds have higher oil content (20-50%) with more than 50% oil yield for *Moringa oleifera* and M. *peregrina*.

Seeds are used as water coagulant in most of the countries across sub-Saharan Africa, particularly, in Sudan. One *Moringa oleifera* seed weighs approximately 0.2 grams. One seed treats a litre of turbid water with approximate turbidity of 100 – 500 NTU. This implies that 0.2 grams of *Moringa oleifera* seed treats one litre of water. Two hundred grams of seeds is required to treat a thousand litres of water per day. This implies that a million seeds can treat a million litres of water per day. In Nigeria and Cameroon, one tree of *Moringa oleifera* produces on an average 30 pods with each pod bearing approximately 10 seeds. Approximately, 33 *Moringa* tree stands can produce 10,000 seeds. It is therefore, estimated that two hundred (200) tree stand can occupy one hectare of land implying that 1.6 hectares of land containing *Moringa oleifera* plant can treat a million litres of water per day.

The leaves of *Moringa oleifera* is used as vegetable and also as an ornamental plant in Saudi Arabia and the middle east region (Munyaiziza and Sarwatt, 2003). Chemical analysis shows that the *Moringa oleifera* seeds have high levels of oleic acid (70.52%), gadoleic acid (1.5%) palmitic and (8.9%). Other constituents include alpha and delta tocopherol values of 145 and 66 mg/kg. Tocopherol is commonly called vitamin E and is very important component of body creams and lotions. Apart from toning the skin, it guards the skin against skin cancer. Similarly, vitamin E is also, therapeutically, useful in improving fertility in humans and averts the formation of prostate cancer (Burkill, 1985a). Sterols greater than 1.55% have been reported and include; 24-methylenecholesterol, campesterol, stigmasterol and D5-avenasterol. The saponification value (185), stearic acid (3.82%), and Iodine value of 69.6 according to Tsaknis (1998) all support that the oil possess good cosmetic value. The peroxide value of 0.4 meq/kg signifies that the oil can preserve longer without fast deterioration. The refractive index at 40% is 1.460, while density at 24% is 0.906, implying that that the oil cannot easily go rancid.

### 1.6 Production vis-a-vis International Trade

The seed oil of *M. oleifera* has been recognized as the most important product, as observed by Plant Resources of Tropical Africa, PROTA. (Polyprasid, 1993 and Tsaknis,
1998), and is generally called “Ben oil”. This oil has been in trade since ancient times in Egypt and other countries in the Middle East for cosmetics.

Other *Moringa* species produce high oil content, more than 50% oil, which is higher than its relative *M. oleifera*. The oil carries various trade names like; ben oil, *Moringa*, behen, baq or horseradish tree oil. Ben oil is produced in large quantity in India where *Moringa* plantations exist and is exported to Parisian perfume industries. VEGIndia exports is a renowned exporting company of ben oil, and thus engage in exploiting products for medicinal value such as the use of the root extracts of *Moringa oleifera* in the treatment of ovarian cancer (Tsaknis, 1998).

1.6.1 Ecology of *Moringa oleifera*

*Moringa oleifera* is, naturally, distributed in the sub-arid or arid region running from North and East Africa to Israel. It has been reported specifically in Somalia, Sudan, Egypt, Libya, Yemen, Oman, Saudi Arabia, Palestine and Israel. The plant tolerates low rainfall and saline soils and is very drought tolerant.

For instance, in Ein Gedi Nature reserve in Israel, it grows well with a number of drought tolerant plants such as Sodom apple (*Calotropis procera*), balanites (*Balanites aegyptiaca*) commicarpus (*Commicarpus plumbagineus*). Flowering maple (*Abutilon hirtum*), Nightshade (*Solanum incanum*), salvadora (*Salvadora persica*), jujube (*Ziziphus spina-Christi*) and Acacia (*Acacia raddiana and Acacia tortilis*). *M. oleifera* as well as these plants thrive above 1000 metres above sea level. It does have a high affinity to natural vegetation formation during spring and in hot desert-like condition. For instance, *Moringa oleifera* has been identified in the Gulf of Oman desert and semi-desert of United Arab Emirates alongside other tree species indigenous to this region such as *Zizyphus spina – Christi, Prosopis cineraria* and *Acacia tortilis*. At Musandem and Hajar Mountains *M. oleifera* grows together with other tree species such as Ficus, *Cordata salicifolia*, *F. johannis*, *Acacia tortilis* and *Prunus arabic* while in Oman’s Batinah coast, it grows as a mixed crop with thorn trees such as *Acacia tortilis* and *Prosopis cineraria*. These trees also thrive well in more sandy soil. In the Ein Gedi reserve, at the eastern periphery of the Judean Desert, it grows side-by-side many plants including sodom apple (*Calotropis procera*), balanites (*Balanite aegyptiaca*).

1.6.2 Artificial Propagation of *M. oleifera*

Like other *Moringa* species, *M. oleifera* can be propagated through seeds and cuttings. These two methods have been employed to raise *Moringa oleifera* in many countries particularly in India, Tanzania Eastern and northern African countries. There is an
advantage using seeds for propagation though, in that the resulting seedling is equipped with a taproot, which is vital for survival especially in areas with marginal rainfall. Trials on artificial planting of *M. oleifera* are popular compared to other species. These trials consisted of nursery experiments on seed germination, growth and survival. The Sudan experience revealed that seed germination was sensitive to temperature and that seedling growth and survival was negatively affected by exposure to full sun radiations and heat. Exposure to full light inhibited seed germination and consequently growth. Half shade was observed as ideal condition at the nursery stage. Previous corroborative studies, for instance, with *M. oleifera* and *M. stenopetala* showed 40-52% germination rates recorded in full light conditions as opposed to 92-94% germination recorded in half shade condition.

There are *M. oleifera* plantations across Africa. *Moringa oleifera* seeds require little or no pre-treatment prior to germination with viability rates for fresh seeds having been reported to be up to 80% dropping to approximately 50% after 12 months storage. Seeds may be sown directly or in seedbeds with transplanting after 2 or 3 months. With regard to seedling transplanting, early or rather hasty transplanting has been discouraged. In line with this, the transplanting of five-month-old seedlings has shown 100% survival. This satisfactory growth of the transplanted seedlings depends mainly on suitable spacing and adequate watering. In addition, irrigation at a rate of 800m$^3$ per acre per month has been observed to sustain survival in many rural areas in Sudan. From both seedlings and cuttings, the *Moringa* tree in general grows at a remarkable rate; three to four meters annual growth in height has been recorded. Cuttings have been primarily utilized for the establishment of live fences and further observations show that branches between 1 and 1.5m in length performs well. With full growth, first fruits may be expected within 6-12 months of planting out. A single tree may produce between 400 to 1000 pods annually depending on the variety and the extent of fertilization and ecological factors. In order to promote branching, pollarding or pruning has been recommended before harvesting. This increases pod production, facilitate harvesting and keeps the tree at a considerable height easy to manage.

### 1.6.3 Diseases and pests

Scanty information exists on the pest of *M. oleifera* and is expected that further elucidation will occur as domestication intensifies. However, it is known that this species play host to *Lycus* (xylotrogus) *africanus*, which a wood is damaging coleopteran that has also been reported in Israel. This fungus attacks a wide range of host (both native and exotic species) such as *Delonix regia*, *Coffea arabica*, *Coffea robusta*, *Grevillea robusta* as well as *Prunus americana*. Control measures have been employed to reduce the import of the host exotic timbers that carry spores of these fungi.
1.6.4 Conservation Strategies for *Moringa Oleifera*

The population of *M. oleifera* has witnessed a steady increase due to increasing awareness of the diverse applications of the plant. The major challenge at the moment lies on how to increase large scale application of the seeds in large-scale water treatment. There are many nurseries on *Moringa oleifera* across sub-Saharan Africa with view to propagating *Moringa* for vegetable production, animal feed and fodder, oil for culinary and medicinal applications. Since the year 2000, over 3.5 million seedlings have been planted in Egypt, Israel, Saudi Arabia and that entire region. Conservation efforts have been deployed in collaboration with the United Nations (UN) agencies, government research institutions and the indigenous people. Furthermore, a number of research projects have been initiated with an overall purpose of understanding the biology, ecology and the economics of *M. oleifera*. Data emanating from such projects could be a valuable resource for the sustainable management of the species under natural conditions, its propagation under artificial conditions, and also for utilization by indigenous people as well as for global trade.

1.6.5 Breeding

This is another possible mechanism to improve upon the survival of *M. oleifera*. Given the increasing value of *M. oleifera* and the nature of the environment in which it grows, this species need to be studied in terms of its breeding characteristics. Breeding for increased trunk size and reduced height may probably lead to a higher yield; this is because the tree is slender and prone to fall if harvest is done after climbing. In addition, breeding for increased branching would most likely go hand in hand with increase in the number of leaves and seeds, while breeding for increased oil production or a hybrid of *M. oleifera* and *M. peregrina* for instance may generate fast growing (as *M. oleifera*) and high oil yielding (as *M. peregrina*) variety. Breeding for better adaptation in the temperate region may be explored. A careful selection of clones and the development of hybrids are considered essential to significantly attain the full potential of both *Moringa* species especially with *Moringa oleifera* that is well known. Such breeding experiments, however, are yet to be mounted.

1.6.6 Genetic Resource base for *Moringa oleifera*

The need to generate a genetic (germplasm) resource base is imperative. One of the most outstanding pressures on *M. oleifera* in the future could be over harvesting for fuel wood. This has been observed with *Moringa peregrine* in Israel and Egypt. In order to avert this and subsequently restore the population of this species, botanical gardens in Israel are now creating seed banks of species of *M. peregrina*. This has not been observed with *Moringa oleifera* yet.
Table 4: Attributes of *Moringa peregrina* compared with some common *Moringa* species

<table>
<thead>
<tr>
<th>Parameter</th>
<th><em>M. oleifera</em></th>
<th><em>M. peregrina</em></th>
<th><em>M. stenopetala</em></th>
<th><em>M. longituba</em></th>
<th><em>M. drouhardii</em></th>
<th><em>M. valifolia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetable</td>
<td>Asia, Africa, America, Green pods, flowers roasted.</td>
<td>Not known nor reported</td>
<td>Not known nor reported</td>
<td>Not known nor reported</td>
<td>Not known nor reported</td>
<td>Not known nor reported</td>
</tr>
<tr>
<td>Oil for cosmetics and cooking</td>
<td>Asia, Africa and Madagascar (seeds used)</td>
<td>Near East</td>
<td>Not known nor reported</td>
<td>Not known not reported</td>
<td>South Madagascar</td>
<td>Not known not reported</td>
</tr>
<tr>
<td>Water coagulant</td>
<td>Sudan, Indonesia (seeds used) (basically Africa, Asia)</td>
<td>Laboratory</td>
<td>Not known not reported</td>
<td>Not known not reported</td>
<td>Not known not reported</td>
<td>Not known not reported</td>
</tr>
<tr>
<td>Honey clarifier</td>
<td>Sudan (seeds)</td>
<td>Not known not reported</td>
<td>Not known not reported</td>
<td>Not known not reported</td>
<td>Not known not reported</td>
<td>Not known not reported</td>
</tr>
<tr>
<td>Medical value</td>
<td>Asia, Africa Central America (all parts)</td>
<td>Near East up to Sudan</td>
<td>Not known not reported</td>
<td>Not known not reported</td>
<td>Bark/roots Madagascar</td>
<td>Not known not reported</td>
</tr>
</tbody>
</table>
Chapter 1

1.7 *Moringa* Phytodisinfectant—Sand Filter Water Treatment

The sand filter is inexpensive, easy to install and has been used as an effective means of treating water for more than a hundred years (Bart *et al*., 1997; Muyibi and Evison, 1995a). A combination of biological and mechanical processes is responsible for this. When water is poured on the top layer of the filter bed, the organic material it carries is trapped at the surface of the fine sand forming a biological layer (Schmutzdecke). This biofilm layer matures over a period of three weeks or more depending on the volume of water, the amount of nutrients and microorganisms in the water (Barth *et al*., 1997). Sand filter has proven to remove more than 96% of faecal coliforms, 100% of protozoa and helminthes (Stauber *et al*., 2009), and 50-90% of organic and inorganic toxicants from water (Bart *et al*., 1997). However, the formation of the biofilm layer is crucial and for water with low turbidity effective filtration is compromised (Barth *et al*., 1997). Furthermore, the filtration rate is very slow (0.1 to 0.3 m\(^2\) per hour) and takes more than three weeks for effective filtration (Lillihook, 2005). There is a need to revisit our roots, study this indigenous system and improve on them. On the other hand, natural coagulants and disinfectants have been used for many centuries in Africa to treat water (Jahn, 1986; Pollard *et al*., 1995; Okuda, 1999 and Prasad, 2009). For instance, seeds from *Moringa oleifera* have proven effective in water treatment (Sutherland *et al*., 1994). There is a growing evidence suggesting that extracts from *Moringa* seed possess both coagulating and antimicrobial properties and safe for human health (Eilert *et al*., 1981; Gassenschmidt *et al*., 1991; Gassenschmidt *et al*., 1995; Folkard *et al*., 1993; Okuofo, 1995; Okuda *et al*., 2001) as well as heavy metals removal from water (Kumari *et al*., 2006). Fig. 3 below shows the seeds of *Moringa oleifera* - the part that is used as a coagulant in water treatment and the anatomy of the plant showing the pods bearing the seeds. *Moringa oleifera* belongs to the family of Moringaceae, order brassicales and in the division magnoliophyta. Generally the division comprises of one genus and thirteen species. These include: *Moringa arborea*, *Moringa bornziana*, *Moringa concanensis*, *Moringa drouhardii*, *Moringa hilderbrandtii*, *Moringa longituba*, *Moringa ovalifolia*, *Moringa pygmaea*, *Moringa rivae*, *Moringa ruspoliana*, *Moringa Stenopetala* and *Moringa oleifera*. *Moringa* can grow in areas of limited rainfall and poor soil. The plant has five petals sepals, five antesepalous stamens and five antepetalous stamens with monotheral and bisporangiate anthers. The seeds have a fast germination rate under poor light conditions. It can be sown through the seeds as well as cuttings from the stem of between 1 and 1.5m in length. A single tree may produce up to 400 to 1000 pods annually depending on the variety and prevailing ecological factors. In villages in Benue state of Nigeria, more
than 95% of the houses have a *Moring* tree. An average of 20 seed bearing pods was observed with an average of 12 seeds per pod (Yongabi, 2004) One seed treats a liter of turbid water (with a turbidity of range of 100 to 200NTU) (Pritchard et al., 2007).

![Image](a)

(b)

**Figure 4:** (a) *Moringa* seeds and (b) plants

Folkard *et al.* (1993) reported that while aerating well water in rural areas of Sudan for reduction of carbon dioxide prior to softening, numerous complaints of red water in hot water systems were received even when aerating was continued and carbon dioxide neutralized with lime in the regular plant treatment process. These complains ceased and did not reoccur as *Moringa* seeds were used. *Moringa oleifera* is a tropical plant in the family of Moringaceae native to India but found all over the world (Jahn, 1986). It has been described as a miracle tree because the entire plant has amazing uses (Fuglie, 2001). The leaves contain vitamin C seven times more than in oranges, Vitamin A four times more than in carrots, calcium four times more than in milk, potassium three times more than in bananas and protein two times more than in yogurt (Fuglie, 2001). Furthermore, aqueous extract of the seeds contain antimicrobial activity against *Pseudomonas aeruginosa* (Armando *et al*., 1991) and against a wide range of gram negative and gram positive bacteria and fungi (Raheela *et al*., 2008).

Previous studies demonstrate that *Moringa* seed possess a bioactive protein (Moringa Coagulant Protein-MOCP) responsible for turbidity removal from water (Jahn, 1986; Sutherland *et al*., 1994; Muyibi *et al*., 2003).

less than 10DA

(Muyubi *et al*., 2003)
However, a lot of constraints exist on a commercial application of *Moringa* seed extracts in large scale water treatment systems (Sutherland *et al.*, 2004). *Moringa* seed treated water cannot store for more than 24 hours. This is because *Moringa* seed generate a lot of organic carbon in water thereby stimulating regrowth of bacteria after 24 hours. Treating large volumes of water requires a longer hydraulic retention time and using *Moringa* as a coagulant is not possible. This has provoked several studies with interest in isolating and purifying the bioactive coagulant protein (Olsen, 1987; Jahn, 1986; Muyibi and Evison, 1995; Ndabigengesere *et al*., 1995; Okuda, 2001; Kebreab, 2005 and Amir *et al*., 2010). These studies have been carried out to clarify the performance of MOC extracted with different methods especially with salts (Tetsuji *et al*., 2001). Most of these studies, however, suggest that the active coagulant ingredient is a protein (Kebreab, 2005 and Amir *et al*., 2010) but other reports exist to suggest that the coagulant ingredient is a water soluble lectin and antioxidant (Santos *et al*., 2005) and Suarez *et al*. (2003) reported a plant peptide. Okuda and coworkers in their studies demonstrated that it is neither a protein nor a polysaccharide but an organic polyelectrolyte (Okuda *et al*., 2001). Although *Moringa* seed is a good coagulant, like the slow sand filter, is only very effective with highly turbid water (Muyibi and Evison, 1995). Furthermore, more studies on the disinfection property of *Moringa* seed extracts are necessary. There have been more studies *Moringa* coagulant activity for three decades (Jahn, 1986; Olsen, 1987; Sutherland *et al*., 1994; Warhurst *et al*., 1996; Muyibi *et al*., 2002a; Muyibi *et al*., 2003; Kebreab *et al*., 2005 and Prasad, 2009) but these studies are increasingly tailored toward the isolation and purification of the active components with so far little field applications to treat the soaring water crisis in Africa (Yongabi, 2009).
It is believed strongly that integrating *Moringa* seed powder into a disinfectant–sand filter bed could treat any type of turbid water with a faster flow rate and shorter residence. It is believed that this could be cost effective.

**1.8 AIMS/OBJECTIVES OF THE PROJECT**

The ultimate purpose of this research work was to evaluate the disinfection efficiency of a *Moringa* disinfectant sand- filter system for water purification.

The specific objectives of the project are summarized into two parts as follows:

**Part 1:**

1. To test a *Moringa* sand filter column for its disinfection activity using synthetic contaminated water using *E. coli*, *Aeromonas hydrophila*, and total heterotrophic aerobic bacterial from soil.

2. To test a constructed *Moringa* Sand filter column for its coagulant activity using a synthetic bentonite- turbid water.

3. To survey and document indigenous knowledge and study the use of indigenous medicinal plants with a history of use in purifying water for their coagulant and disinfection ability using surface contaminated water.

4. To carry out a bioassay guided cold Solvent extraction using solvents of varying polarities on *Moringa* seed powder and test its in vitro antibacterial activity using *E. coli* (ATCC11775). Strain (indicator of faecal contamination of water) and *Aeromonas hydrophila* wild strain (known to resist chlorination). Against aluminum sulphate and sodium hypochlorite controls. Phytochemicals screening and chromatographic analysis were done to elucidate possible bioactive disinfectant ingredient in *Moringa* seeds.

After proof of concept in part 1 experiment, the objectives of Part 2 were:

1. To evaluate the microbial content of surface water (stream) at Bambui and Mile 6 Mankon - a source used for household chores in Bamenda, Cameroon for total bacterial counts, total fungal counts, *E. coli* and coliform counts.
2. To set up a pilot low-cost disinfectant–sand filter drum of 200 litres capacity (That can generate at least 125 liter of clean water a day) and use the same turbid surface water source in Bambui and Mile 6 Mankon in Bamenda, Cameroon to test its disinfection and coagulant efficiency using total bacterial counts, *E. coli* counts, coliform counts, total fungal counts, pH, and turbidity, measured both before and after filtration.

### 1.8.1 Significance /Contribution to the discipline

Integrating *Moringa* in a disinfectant-sand filter system, the short falls of both the sand filter alone and *Moringa* would have been solved. Several studies on the coagulant potential of *Moringa* seed exist with few studies on its disinfection potentials. *Moringa oleifera* has a double advantage over aluminum sulphate because it possesses both phytochemicals, nutritional and antimicrobial properties (Dalen *et al.*., 2009; Yarahmadi *et al.*., 2009). Another advantage is that activated carbon from the seed husk adsorbs heavy metals from water (Warhurst *et al.*, 1996). *Moringa* coagulant protein is less than 12 kildaltons with a net positive charge that possess 99% coagulant activity. It is not clear if the same protein has disinfection activity but the antimicrobial activity from the seeds extract has been reported (Yarahmadi *et al.*, 2009; Dalen *et al.*, 2009). This study could provide some useful insights into the disinfection activity of *Moringa* seed extracts. The use of *Escherichia coli* spiked water to study the disinfection activity of *Moringa* sand filter bed is novel. *E. coli* is an indicator of faecal contamination of water and is being used in routine monitoring of water quality (WHO, 2006). Furthermore, *Aeromonas hydrophila* is an organism widespread in water distributions and is becoming resistant to chlorination (Pritchard *et al.*, 2009). *Aeromonas hydrophila* is a gram negative, catalase and oxidase positive, non-motile bacteria with a high resistance to many antibiotics (Rogo *et al.*, 2009). Studies on the antibacterial activity of *Moringa* seed extracts using different polarity based solvents could a source of alternative antibiotic and anti-infective against *Aeromonas hydrophila*. There is no literature on the use of *Moringa* extracts on *Aeromonas* strains isolated from water. The choice of extraction using 5 solvents ranging from non-polar hexane to water could provide information on the best extracting technique for bioactive components from *Moringa*. The seeds as well as the shells will be used for extraction, extract yield and bioactivity compared. The design of the rig to be used in this research was 0.5 m bed depth and was packed with fine sand of particle distribution size range from 0.1mm to 0.3mm in diameter. A bed of pulverized *Moringa oleifera* seeds of 20mm was superimposed on the fine sand layer. Furthermore, filtered was collected at different bed depths at every 12.5 cm and at
four different positions. This provided information on appropriate depths for filtration, flow rates and residence time. This set up was unique in that a *Moringa oleifera* provided primary coagulation and flocculation before filtration with fine sand layer. There was no formation of biofilm layer as it would normally occur on a slow sand filter. This approach reduced the residence filtration time and flow rates were increased. This provided 100% disinfection and turbidity removal with less sludge volume. Providing clean drinking water at household level in just a day at low cost with little maintenance services could be a great contribution to sustainable development.

The outcome of the microbial analysis of water source in Cameroon provided useful data on the water contamination level. Furthermore, data on other local plants used in water treatment was made available for future studies. This may trigger interest in bottom top approach in seeking solutions to contemporary problems. It is also expected that industries and companies could scale up the disinfectant – sand – filter system and adapt it to treat and dispose wastewaters of all types at low cost.

**1.8.2 Theoretical framework and Methods**

The slow sand filter relies both on physical and biological activity of the biofilm layer. The organisms that make up the biofilm come from the turbid water (Kebreab, 2005). These organisms secrete antimicrobial substances that in turn predate on the pathogens present in the water. This takes about seventeen days implying that users have to wait for this length of time to get clean water. In very highly polluted water the sand filter is incapable of filtering off viruses due to their very small size (less than one micron). Furthermore, the viruses that are filtered off are not killed but attached on to the surface of the biofilm. Once attached, antimicrobial substances produced by the biofilm lyses the virus. This implies that the sand filter must have a good biofilm to be reliable. Boiling and post filtration chlorination has been recommended after filtration of water with a slow sand filter (Barth, 1997; Okuda et al., 2001). Furthermore, the efficiency of the conventional slow sand filter depends much also on the particle size distribution of the sand, the ratio of the surface area of the filter and the flow rate through the filter bed. The filter bed has to be kept with water all the time as this prevents drying up and cracks on the biofilm. Pretreatment of water with *Moringa* coagulant before filtration on the sand bed would mean that this traditional sand filter operational mechanism would not be the case. The traditional bed depth range from 0.5 m to 1.5 m is required for a good sand filter. The greater the bed depth the more efficient the filtration (Lillihoek, 2005). The addition of a pretreatment with *Moringa* could alter these design parameters. Clogging of filters and frequent backwashing of the sand filter may also be less frequent with an additional pretreatment with a coagulant (Muyibi et al., 2001).
coagulation mechanism of *Moringa oleifera* has been explained in different ways: by adsorption and charge neutralization (Kebreab *et al.*, 2005), interparticle bridging (Muyibi *et al.*, 2001). In this research, slow sand filter technology with the addition of a coagulant and disinfectant of plant origin may possess the following properties; the formation of bio-layer not necessary as filter could be used at once after installation, 80 to 90 litres of water per hour of clean water is expected, does not require post filtration chlorination, adopts a holistic combination of processes such as coagulation, adsorption, predation, disinfection and mechanical filtration. The possible advocacy to replace the use of aluminum sulphate, a chemical coagulant, and chlorine, a chemical disinfectant, by creating an inventory of plants that have been used in rural Africa and integrating them with a slow sand filter system to treat water is novel. The assessment of a *Moringa* disinfectant sand filter drum for its efficiency in generating clean water in a day rather than so much focus on isolating the active ingredient for more than three decades as previous researchers are doing (Jahn, 1986; Olsen, 1987; Sutherland *et al.*, 1994; Warhurst *et al.*, 1996; Muyibi *et al.*, 2002a; Muyibi *et al.*, 2003; Kebreab *et al.*, 2005 and Prasad, 2009) provides a paradigm shift in plant coagulant and disinfectant research, thus a strong theoretical frame work for this research. In this research work, *Moringa* seed powder of particle size between 0.001mm to 0.1 mm will be integrated onto fine sand with particle distribution size range of 0.05 to 0.5 mm as indicated in Fig.6 below.

*Figure 6: Particle size distribution curve for Moringa (1) and sand (2)*

### 1.9 Indigenous Knowledge, Science and Technology
Every society evolves with its own problems but there could be latent solutions behind the problem. In this research, it is believed that the solutions to the water crisis lay in the appropriate exploitation of the inherent resources through an appropriate application of science and technology (Olsen, 1987). The documentation of indigenous plants with potentials for water treatment in local Bolivia where poor water quality was a problem has been reported. Cactus was reported as an indigenous coagulant in Bolivia (Zhang et al., 2006). Such studies are very necessary. This study generated a catalogue of plants used in Cameroon to purify water through visits to the rural communities and a prioritization system to select the most suitable plants took into accounts its availability, purification potential on water, frequency of use by the different local communities, and available published reports. The plants with considerable coagulant and disinfection potentials were integrated on to a slow sand filter system.
Developing countries continue to face potable water supply problem because of inadequate financial resources (Muyibi and Evison, 1995). The cost of water treatment is increasing and water quality remains poor (Jahn, 1988; Amir et al., 2010) Suspended and colloidal particle load is high mainly caused by land development and high storm runoff during prolonged wet season. Approximately, 1.2 billion people lack safe drinking water and more than 6 million children die from diarrhoea each year. Waterborne diseases caused by viruses, bacteria, protozoa and other microorganisms is associated with this outbreaks (McConnachie et al.,1999) many synthetic coagulants and disinfectants are widely used in conventional water treatment processes (Okuda et al.,2001b). Aluminium and Iron salts are the most commonly used coagulants (Muyibi and Evison, 1995) Halogenated compounds such as chlorine and fluorine are used as disinfectants (Muyibi et al., 2002a, 2002b). However, aluminium sulphate is linked as a precursor of Alzheimer’s disease (Crapper et al., 1973; Martyne et al., 1989) and chlorine produces trichloromethane, a precursor of cancer (Dearfield et al., 1964; Mallevialle et al., 1984; Okuda et al., 2001b). These synthetic coagulants and disinfectants are imported to Africa at high cost (Kebreab et al,2005). In Nigeria, some state governments spend more than 50% of their annual budget in importing water treatment chemicals (Muyibi et al.,2003) as well as in Malawi (Pritchard et al.,2009). Other conventional water treatment technologies such as Ion exchange, Membrane
filtration, UV sterilization and reverse osmosis are very effective (Prasad, 2009). However, the high cost of installation, high energy inputs and maintenance cost renders it impossible for use in developing countries (Muyibi et al., 2002a, b). The slow sand filtration has been traditionally used in many developing countries for more than 150 years (Garry et al., 2002). Despite its current and historical importance, the fundamental physical and biological mechanisms controlling water purification and head loss development are poorly defined (Corinne et al., 2002). For instance, mycobacteria have been detected in tap water systems filtered using slow sand filters in France (Corinne et al., 2002). Additionally, the flow rate (0.1 – 0.3 m/h) is too slow. After installation, it requires more than three weeks to obtain treated water (Garry et al., 2002). Recently there has been increased interest in the use of natural coagulants and disinfectants in water treatment (Schultz and Okun, 1983; Kebreab et al., 2005; Pritchard et al., 2009). Among these, *Moringa oleifera* seeds are quite commonly used as a primary coagulant in water treatment application (Muyibi et al., 2002a, b; Kebreab et al., 2005). A review of slow sand filtration, synthetic coagulations and disinfectants was carried out. Also the problems and challenges involved with the application of *Moringa oleifera* coagulant and disinfectant, as well as prospects of a sustainable low cost water treatment alternative, has been investigated.

### 2.1 Introduction/Background

The provision of safe potable water to communities in the world is a necessary condition for development (Reid, 1982; Schultz and Okun, 1984; UNICEF, 2009). This is one of the central objectives of the World Health Organization (Pollard et al., 1995; WHO, 2006). Safe drinking water and adequate sanitation are essential for human health and dignity, yet 1.2 billion people do not have access, with close to 2.5 billion lacking adequate sanitation facilities (UNEP, 2002; UNESCO, 2007 and UNICEF, 2009). More than 6 million children die each year due to diarrhoeal related diseases as a result of contaminated water (McConnachie et al., 1999 and Yarahmadi et al., 2009). An average of 125 litres of clean water is needed per person a day, yet in Africa a majority of people cannot boost of 25 litres of clean, disease-free water. Developing countries pay a high cost of importation of synthetic chemicals including polyaluminum chloride, alum and chlorine (Ghebremichael et al., 2004 and Lilliehook, 2005). For example, Malawi has 52.4% of its population that live below poverty line (Pritchard et al., 2009). For this reason, these countries need low cost technologies requiring minimal maintenance and skill (Yarahmadi et al., 2009). Polyaluminum chloride is widely used water treatment but polyaluminum chloride and alum add impurities during water treatment such as epichlodine which is a potential carcinogen
Additional, Aluminium is regarded as an important poisoning factor in dialysis encephalopathy (Yarahmadi et al., 2009). Aluminium is also one of the factors which have been linked to Alzheimer disease (Okuda et al., 1999). Furthermore, the application of aluminium in water treatment reduces pH (Muyibi and Alfagara, 2003) and thus requires extra cost to stabilize pH with calcium oxide (Okuda et al., 2001a). Some synthetic organic polymers applied in water treatment such as acrylamide have been reported to cause neurotoxicity and are potentially carcinogenic (Muyibi and Alfagara, 2003). Slow sand filter technology, one of the earliest forms of water treatment, is effective, cheap and safe (Garry et al., 2002). However, the flow rate is slow and frequent backwashing is required when filtering water with very high turbidity (Garry et al., 2002). The use of natural macromolecular coagulants such as Moringa oleifera seeds has gained interest in the last three decades. This has attracted attention of many researchers because of its abundance, low cost, biodegradability and high efficacy in water treatment (Yongabi, 2004; Kebreab et al., 2005; Yarahmadi et al., 2009; Pritchard et al., 2009).

For this purpose, a strong need to develop a low cost sustainable water treatment alternative using Moringa oleifera and slow sand filters was considered.

2.1.1 Overview of the importance of Water

Approximately 75% of the human body weight is made up of water. Water has a wide application in health, agriculture and irrigation, social as well as environmental areas. Water is essential for human survival. It has been reported that the total amount of water in the world is about 1400 million cubic km (= 10^{18} tonnes) and remains constant (Adegbola, 1987). More than 97% of this total volume is seawater, of the rest, 22% is ground water and 75% is ice locked away in the glaciers and the polar and Antarctica ice caps. This leaves less than 1% of the supply of fresh water with half of this found in rivers, lakes, and swamps and mostly polluted. In Sub-Saharan Africa for instance, 95% of the surface water is considerably polluted (Adegbola, 1987; Muyibi and Evison, 1995a; Pollard et al., 1995; Muyibi et al., 2002; UNICEF, 2009).

According to Cofie et al (2003) 90% of water in India and in Bangladesh is polluted (Talukder et al. 2007). Additionally, most surface water in developing countries remains suspicious in terms of quality as noted by Hart et al (2005) and Amir et al (2010). The ever-increasing prevalence of endemic diseases like diarrhoea, dysentery, amoebiasis hepatitis, typhoid, jaundice and more may be suggestive of the severe exposure to harmful effects of water contamination in developing countries (Sciban et al, 2009). In some developed countries water borne diseases are still prevalent despite the application of advanced technologies for treatment. Poor water quality has been reported in rural areas in Australia.
Chapter 2

(Thurman et al., 1998) and in the United States (Hegarty et al., 1999). Occurrence of *Helicobacter pylori* has been reported in surface water in some parts of the United States. Similar observations in Chile and in Peru have also been reported (Medall et al, 1992; Hopkins et al, 1993 and Hulten et al, 1996). The disposal of garbage, sewage and industrial effluents into rivers has been attributed to high level of pollution (WHO, 2006). Apart from microbial contamination of water, chemical pollutants also affect the environment. Toxic substances like aldehydes, ketones, amines and carboxylic acids present in water in very small amounts deplete the dissolved oxygen and alter the survival pattern for aquatic life.

2.1.2 Sources of water

Ground, surface and rain waters are often the major sources of water in African communities. Ground Water is often the most appropriate source of water for drinking as long as it does not contain high mineral content. Ground water could be extracted through wells or bore holes. Surface Water requires treatment to make it safe for human consumption. Surface water is almost always contaminated by people and animals who defecate in or near the water. The water is obtained from streams, lakes, ponds etc. Rain Water is generally considered as pure. It can be collected in large storage basin and/or smaller containers. However, rain water collected in dirty or unclean containers has to be treated to make it safe for drinking (Olsen, 1987 and Pritchard et al, 2009).

Water gets contaminated in so many ways, particularly through anthropogenic factors, thereby making it unsafe for consumption (Adegbola, 1987).

2.1.3 Microbes in surface water, public health implications

WHO (2006) reported that more than 80% of diseases in the 3rd world are due to poor water quality and sanitation. There are many disease-causing microbes found in contaminated and polluted water which are harmful to human beings when they are consumed in drinking water. Many of the diseases that cause illness and subsequent death of humankind are related to water in one way or the other. Some of the water-related diseases are outlined:

Water-borne Diseases: These are diseases, which can affect a person when contaminated water is drunk. They include cholera, dysentery, diarrhoea, typhoid, hepatitis and anaemia due to worm infestation according to Jyoti et al (2010).

Water-based diseases: These are disease caused by the use of contaminated water to bath or when in contact with the skin or eyes. They include ringworm, bilharziasis, conjunctivitis and dracunculosis (guinea worm) according to Wohlsen et al (2008).

Water-wash disease: These are diseases caused as a result of lack of bathing i.e. there is a need to wash the body with water to avoid them. They include scabies, trachoma etc.
Water-related Insect Vectors and their Diseases: These are insect vectors which breed in stagnant water and which when they bite a person can cause disease. The insect vectors include flies which cause filariasis, particularly onchocerciasis, and finally mosquitoes that cause malaria and yellow fever (Cheesbrough, 1984).

Continuous studies on the incidence of these waterborne diseases and their etiologic agents in surface water and development of sustainable mitigation strategies remain exigent for Africa (Yongabi, 2009).

2.1.4 Effects of untreated wastewater on drinking water sources

Wastewater (also termed sewage) is said to be the water-borne waste of a community. It may be a combination of solid waste from domestic use, commercial building, industrial plants and institutions all combined with ground water, surface water and run-off water (Bedwell and Goulder, 1996; Bastos et al, 2004; Mull and Hill, 2009 and Jyoti et al, 2010).

Wastewater in most cases is discharged into other water sources contaminating and polluting it. This wastewater, generally, contains a wide spectrum of pathogenic organisms, particularly etiologic agents for enteric diseases in man according to Cheesbrough (1984). The spectrum of diseases and their causative organisms have been highlighted above but suffice to mention that the strains greatly vary from one community in Africa to another as well as the source of water. For instance, cholera caused by vibrio cholerae, typhoid fever caused by Salmonella typhi, bacillary dysentery caused by Shigella species amongst others are very predominant in sub-Saharan Africa (UNESCO, 2007 and Jyoti et al, 2010). Pathogenic organisms from waste can be broadly classified as bacteria, fungi, viruses, protozoa and helminthes. A number of diseases, and disease causing agents, have been isolated from poultry wastes and these include; New Castle disease virus, Chlamydia, psittacosis (conjunctivitis and pneumonia in humans), Erysipelothrix rhusiopathia (causes erysipelas), listeria monocytogenes (listeriosis), Mycobacterium avium, Candida albicans, Aspergillus fumigatus (rhinitis, asthma, and chronic pulmonary disorder) Clostridium (food poisoning and botulinum), Salmonella spp, Bacillus anthracis (anthrax), Brucella abortus (Brucellosis), Leptospirosis, Escherichia coli and bovine tuberculosis (Tappouni, 1984 Pike, 1990; Joyce et al., 1992; McQuigan et al., 1998; Skirrow, 1994; Satory 1998). Toxigenic fungi exist in poultry waste with a remote possibility of transmission under certain changing conditions. Animals are asymptomatic carriers of organisms that can cause disease in other species (Tang, et al 1994; WHO 1982; Atabay et al, 1997; Skirrow, 1994; Weijtens et al, 1997 and Rosa et al., 1998). Hegarty et al (1999) observed in their study no correlation between the presence of Helicobacter pylori and the traditional indicator organisms in water.
supplies. Epidemiological association between water sources and the prevalence of *H. pylori* infection has also reported by several researchers (Klein et al 1991; Remirez-Ramos et al 1994; Mendal et al 1992; Mitchell et al 1996; Goodwin 1993; Hopkins et al, 1993). *H. pylori* infection has been associated with the consumption of untreated well or spring water by children (Madore et al., 1987; Jones et al., 1990; Carballo et al, 1997) with further evidence being provided by culture of *H. pylori* from the faeces of infected individuals as reported by Thomas *et al* (1992) and Arrowood and Sterling (1987).

The research work of Hegarty and co-workers in 1999 indicated that methods for the direct cultures of *H. pylori* from water samples remain elusive. Currently *H. pylori* are observed through the use of a microscopic technique coupled with the use of actively respiring microorganisms binding monoclonal anti-*H. pylori* antibody to detect the organism in a majority of surface and ground water samples in the United States. Additionally, Hulten *et al* (1996); and Forrest *et al*, (1998) used *H. pylori* specific nucleic acid sequence to detect *H. pylori* in water in Columbia, Peru, Sweden and as well as in Sewage in the United States. Other techniques such as PCR and combined fluorescent antibody cyanoditoyl tetrazolium chloride (CTC) staining had been used in addition to enumerate *H. pylori* in an attempt to overcome the phenomenon of viable but non cultivable occurrences (VBNC *H. pylori*) (Annan-Prah and Janc,1998; Josephson et al.,1997; Farouq et al.,1993; Turner et al., 2000). The studies of Hegarty *et al* (1999) and Leclerc et al (2000) also strongly indicated that *E. coli* was not detected in 50% of the samples in which *H. pylori* was detected. This lack of significant association between the presences of *E. coli* for the determination of the potability of water may fail to protect people from *H. pylori* and other specific infections. Such a lack of association may possibly indicate that *H. pylori* may survive longer in a freshwater habitat than *E. coli* or possibility that *H. pylori* is part of a normal flora of many fresh water bodies and can survive in limited nutrients (Herniman et al., 1973; Montecath et al., 1986; Deng and Chiver, 1995).

Bacterial analysis have been the main focus on diseases transmitted by water (Sirrow, 1994; Satory et al, 1998; Smith, 2000) but fungi and viruses are equally of utmost importance and the traditional coliform/faecal indicator tests do not simulate or correlate well with the presence of fungi and other microbial groups in water and waste water (WHO, 1982; Rosa et al., 1998). For instance, *Aspergillus flavus* have been isolated from water bodies where faecal coliform were not detected (Peterson et al. 1997).

Total coliform were found in 85% of the samples containing *H. pylori* (Turner and William, 1999; Molin et al., 1989; Wegelin et al., 1991), however, there should be a careful interpretation of their association. A conclusive report on the limitations of Indicator
organisms as a reflection of the ultimate pollution picture of water has been done by Efstratiou et al (1998). In their studies on the correlation of bacterial indicator organisms with salmonella spp., Staphylococcus aureus and Candida albicans in sea water, total coliform correlated better with salmonellas and staphylococcus aureus than did faecal coliform and faecal streptococci, faecal coliform correlated better with the presence of Candida albicans.

Their studies showed a strong conclusion that total coliform is sufficient to predict the presence of Salmonella and Staph spp in sea water that is moderately polluted. The works of Leclerc et al (2000) also strongly acknowledge the limitation of indicator organism (E. coli and coliform) to confirm the presence of enteric viruses in human faeces, water and sewage. While acknowledging the overall advantages of the use of traditional indicator organisms, bacteria phages detection has been recommended to indirectly track the presence of enteric viruses. Reports exist to show the continuous isolation of pathogenic microorganisms, especially antibiotic resistant strains, from polluted water and wastewater. Sisti (1998) and Sidhu (1999) reported a high incidence of Aeromonas species from influent and effluent of urban waste and water purification plants. Motile Aeromonas spp are ubiquitous in aquatic environments and have also been isolated from sewage, polluted and unpolluted fresh water and drinking water, even after chlorination, as well as mineral water. Aeromonas have been implicated in gastrointestinal infection; disseminating infections in immuno compromised patients with Aeromonas veronii biotype sobria noted as most virulent.

The potential therefore exists for large numbers of oocysts to enter the sewage treatment works from both domestic sewage and from sources such as cattle markets and abattoirs in the west (Madore et al, 1987) and this probably would be worse in developing countries. Livestock animals are probably more likely to be at risk of infection. Cryptosporidium is widespread and second to rota virus as the most prevalent pathogen in outbreaks of diarrhoea in calves. It has been reported that infected lambs and calves shed approximately 10^{10} oocysts daily between 4 to 14 days post infection while levels of oocysts as high as approximately 4000 1^{-1} in some effluent and 13700 1^{-1} in a raw sewage containing slaughter house waste (Madore et al, 1987).

2.2 A REVIEW OF WATER TREATMENT IN NIGERIA AND CAMEROON

Water proceeds through a number of processes from source to user, for example:
Rainwater or water from catchments – lake pumping station – Reservoir – Sedimentation – Addition of lime – addition of Alum – Coagulation (flash tank) –
The two most crucial steps in water purification are coagulation using alum and disinfection. Alum (Al₂(SO₄)₃.18H₂O) is widely used, as are other polyelectrolytes such as ferric chloride (FeCl₃). During coagulation, lime (CaO or Ca(OH)₂) is used for pH stabilization since Alum generates acid water. Coagulation must have a pH range within which the reaction

$$\text{Al}_2(\text{SO}_4)_3.18\text{H}_2\text{O} + 3\text{Ca} (\text{OH})_2 \rightarrow 2\text{Al} (\text{OH})_3 + 3\text{Ca} \text{SO}_4 + 18\text{H}_2\text{O}$$

is effective. The insoluble aluminium hydroxide forms a gelatinous floc that settles slowly, sweeping out suspended materials. These imported inorganic coagulants require special handling and capital expenditure. As such the final treated water becomes expensive for the locals.

### 2.2.1 Introduction to Water and Wastewater Purification Methods

Water that contains diseases-causing organism is not fit for human consumption. As such it is necessary for water to be purified so as to be made safe for drinking. To achieve this, various methods have been employed. Each method has its own setbacks in terms of efficiency, cost and ecological suitability. There are numerous methods to treat water for it to become safe to drink. The main methods are sedimentation, coagulation, flocculation, filtration and disinfection. Sometimes combining two or more of these methods in a ‘multi-barrier approach’ (CAWST 2009) can improve the quality of the water significantly. As well as removing the micro-organisms that can be harmful to your health, treating water can also improve its taste, odour and colour.

Sedimentation describes the process where suspended particles that are heavier than water fall to the bottom of the vessel under the influence of gravity. The water can then be gently decanted to retrieve the clear water without disturbing the particles at the bottom of the vessel. The particulate matter can then be appropriately discarded. Sedimentation is simple to implement and effective in removing mainly grit and sand particles. Sedimentation reduces the turbidity of the water. Turbidity is a measure of how much suspended particles there are in a given volume of water. Generally, the greater the turbidity the more contaminated the water is. A diagram of the mechanism of sedimentation is shown below.
The amount of sedimentation that occurs is dependent on the size of the particles and how long the water is allowed to stand for. Larger particles settle out of the water easier than smaller particles. The longer water is allowed to stand, the more particles fall to the bottom. However, as shown in Figure 8, smaller particles often remain suspended in the water regardless of how long the water is allowed to stand for. This is due to the strong degree of repulsion between smaller particles.

A coagulant can be added to the water to remove the repulsion. This process is called coagulation. Coagulants can be derived from both chemical and natural sources.

Coagulation

\[ \Delta = \text{Coagulant} \]

Flocculation

Gently agitate

Sedimentation

\[ \text{Floc} \]

One of the most common chemical coagulants used in water treatment is alum. The contaminated water containing the coagulant can be stirred gently to promote to formation of flocs. Flocs are clusters of particles that have been allowed to form due to the addition of coagulant. These flocs are then eventually large enough to settle to the bottom of the water.
containing vessel. This process of agitating the mixture gently is called flocculation. A combination of two or more technologies is better in a given situation. These Methods include; distillation, ion exchange, carbon adsorption, filtration, ultra filtration, reverse osmosis, electro deionization, ultraviolet (UV) radiation.

Distillation is probably the oldest method employed in water treatment but it requires large amounts of energy and water but it removes a broad range of contaminants. It requires expert training, and careful maintenance to ensure efficiency. Ion exchange is very efficient in removing organic contaminants from water as well, the only set back is that microorganisms can attach to the resins and can thus trigger regrowth, coupled with high operating cost. The carbon adsorption is 99.99% efficient in removing suspended solids, the pressure of millipore membrane filters of 0.22um reduce all bacteria but cannot remove inorganic or colloidal particles (Wegelin, 1987; Shehab, 2003; Dutta Gupta, 2003; Cofie, 2003). Ultra filtration acts as molecular sieve, effectively removing all types of particles and microbes, it produces high quality water with minimal energy input, but is still defective in removing inorganic material, while Reverse Osmosis(RO) can effectively remove all types of contaminants to some extent (particles, pyrogenes, microorganisms, colloids and dissolved inorganics) though the flow rate is limited. Electrode ionization is a technology clone from electro dialysis and ion exchange, it’s inexpensive to operate and absolutely efficient in removing inorganics but the set back is that the water requires pre-treatment. The adsorption of UV light by the DNA and proteins in the microbial cells results in cell inactivation but the method cannot remove particles, colloids or ions.

2.2.1.1 General Methods of Water Treatment Used in Africa

1. Boiling of water for some minutes to kill germs.

2. Sedimentation: Water is kept in a pot and allowed to stand for some time when the sediments settle at the bottom the water is carefully taken into another container for drinking.

3. On mountaintops where cavities are found, water is poured into such cavities where the sun’s rays destroy the bacteria. The water is left for a period of time after which the water is scooped or taken out carefully into a clean container leaving the sediments behind.

4. Using sand/cotton filters where a big collection pot is used and another pot for filtering, with a small hole at the base is placed over the collection pot. In the filter pot cotton material is first placed at the bottom of the pot and over it ‘gravel’s of small sizes are placed and finally clean sand is placed. Water is then poured over these media in the pot and passes into the pot at the bottom. By doing so water is filtered and ready for drinking.
Using clean cloth over a big pot, water fetched is strained, the sediments are separated using the clean cloth and the water collected in the pot is free of sediments.

Collection of rainwater in clean containers. First of all the rain is allowed to fall for some minutes to wash the dust and other contaminants down, then clean containers are placed at the collection points for water collection.

2.2.2 Boiling/heating and its setbacks

This is the most common method of water purification in the rural areas. The organism can be destroyed by boiling the water for several minutes, but this requires much fuel and it is quite a cumbersome task. Some spore formers can also survive boiling. Boiling however cannot take care of colloidal particles. The water will have to undergo filtration but household recontamination of water has been observed (Sam Godfrey, 2003).

2.2.3 Solar-air Treatment (SODIS)

Solar-radiation is known to kill harmful bacteria contained in water. The process involved is taking a transparent glass or plastic bottle and putting water into it, leaving 1/3 of the volume. The bottle is then well capped and the bottle is vigorously shaken. It is left to stand under the full effect of the sunlight from dawn to dusk. Shaking of the bottle is done about four times at regular intervals all through the day to ensure photo-oxidation. At dusk, the water is fully purified and one can then drink it. This lends credence to the observation of Wegelin et al, (1994). A water quality test has been carried out on the water treated using this method and result shows effective purification. This method is effective but requires large solar plants, which are expensive to construct in order to provide sufficient water for a community. In a related supportive study, Smith et al (2000) observed that solar radiation inactivated *Salmonella typhimurium* in water, unfortunately frequent rains and dusty environment may limit this method, besides it’s time consuming for a rural setting.

2.2.4 The Halogens and their application in water disinfection

Chlorine is used in water and wastewater treatment for disinfection, prevention and removal of odour and ions, although principally as a disinfectant. Chlorine was first used for day-in-day-out disinfection of a municipal water supply in America when George and Pandalai (1949) added chlorine of lime to water supply in Jersey City.

Bromine and Iodine are also effective germicidal agents. Their chemistry is similar to that of chlorine although an ionisation constant is involved in this case, thus optimum pH differs (Timothy, et al 1976). Iodine, an effective disinfectant is used widely in swimming pools but not in water and wastewater treatment. This is due to its possible physiological effect on thyroid activity and its relatively high cost (Morris, 1971). Monochloroamines and
iodide, a mixture of halogens has been studied as technique for disinfection (Kinman, 1976). Their combination exhibited faster disinfection than either of them alone. Chlorine dioxide and Bromide chloride are mostly applied in wastewater treatment.

2.2.5 Quaternary Ammonium Compounds and Chlorine Solutions

Quaternary ammonium compounds are potential disinfectants but have not been acceptable for potable water due to relatively high cost, possible toxic effect and objectionable taste. Sodium hypochlorite has been used to disinfect water. This is, however, recommended for emergencies because a continuous intake could be eventually harmful to people due to the other ingredients contained in it. A teaspoonful of NaOCl₃ in a 10-litre bucket disinfects the water in 60 minutes and 2 teaspoons disinfect it in 30 minutes. By mixing 2 litres of bleach with 35 litres of water, chlorine solution is formed which can be used to scrub the walls of newly dug wells and remaining is poured into the well. A similar solution is then made and poured into the well and allowed to stand overnight. The well is then pumped to waste until the odour of chlorine disappears. Chlorine may persist for a week or more depending on the volume of water. However, Godfrey, (2003) expressed worries about appropriate dosing and hazards involved for the rural people when handling chlorine.

2.3 Filtration Process.

Filtration is used to describe water treatment processes that involve physically removing micro-organisms and particles from contaminated water. There are various types of filtration, such as; cloth filtration, rapid sand filtration and slow sand filtration. To perform cloth filtration, a piece of cotton cloth is folded into layers and secured over the mouth of a water storage container. Collected surface water is fed onto the layers of cotton cloth and allowed to pass through into the storage container under the effect of gravity (Dangol and Spuhler, 2011). The majority of micro-organisms are retained on top of or in the layers of cotton cloth as the water passes through. Water that has been filtered by cotton cloth has lower turbidity and amounts of pathogenic micro-organisms than unfiltered water. The cloth filtration procedure is shown in Figure 10.
Rapid sand filtration comprises of two stages; flocculation of the particles and passing the water containing the flocculated material through a bed of coarse sand or gravel. The flocculated material becomes trapped between the sand or gravel particles, while the water is allowed to pass through.

2.3.1 Slow sand filter: Historical evolution

Slow Sand Filter (SSF) is one of the earliest forms of potable water treatment technology, used for more than 150 years (Garry et al., 2002). It was first used in Europe in the 1800s and later in North America, primarily limited to smaller communities in New England (Garry et al., 2002). The efficacy of water treatment using a slow sand filter was demonstrated during the 1892 cholera epidemic in Hamburg, Germany, when the science of microbiology was in its early years of development. As described by Gainey and Lord (1952), the disease outbreak involved two cities; Hamburg and Altona, which both used river Elbe as a source of drinking water. Altona, with its water intake located down river from Hamburg’s sewer outfalls, might have expected to suffer seriously from the outbreak but Altona used slow sand filtration to purify water from the Elbe. Hamburg, lacking slow sand filters bore the brunt of the outbreak, with 8605 deaths. Gainey and Lord (1952) reported the death toll from cholera as 1344 per 100,000 in Hamburg and 230 per 100,000 in Altona. This event illustrates the efficacy of slow sand filters for controlling microbiological contaminants in water.

Slow sand filters were built to serve communities in North America both before and after 1900, but the advent of effective coagulation, sedimentation and rapid filtration resulted in a declining interest in slow sand filtration in North America in the early part of the twentieth century. This changed in the later part of the twentieth century when slow sand filtration was evaluated for removal of viruses, giardia cysts, and cryptosporidium oocysts. A driving force for the re-evaluation of slow sand filtration in the United States was the need for simple but effective water treatment processes for small water systems located in rural
areas. A similar re-evaluation had occurred in China. According to Li et al. (1966), slow sand filtration was used in China in the 1930s and 1940s, but later rapid gravity filtration was preferred due to land requirements for slow sand filters in urban areas. Since 1980, slow sand filtration has been applied in rural areas in China for small scale water treatment facilities (Kagwa, 2001).

2.3.1.2 Sand filter, construction and its mechanism of operation.

A slow sand filter comprises of approximately 1.2m depth of fine sand supported on two or three gravel layers. Slow sand filters can frequently be constructed from locally available materials. The effective size of the sand used in slow sand filters is about 0.2mm but may range from 0.15mm and 0.35mm and with a coefficient uniformity of between 1.5 and 3.0. To contrast from rapid filters, the range of effective size of the sand is 0.35mm to 1.0mm, with a coefficient of uniformity of 1.2 to 1.7. The walls of the filter can be constructed of concrete or stone. Sloping walls dug into the earth, supported or protected by chicken wire reinforcement, and lined with sand–bitumen mixture could be a cost effective alternative to concrete. The inlets and outlets are provided with control devices that keep the raw water level and filtration rate constant. Bottom drains are made of a system of manifolds and lateral pipes. The filtration rate was between 2.5 and 6.0m$^3$/m$^2$/day. As water falls or flows over the filter bed it percolates through the sand grains, where the disease-causing organisms die out. Clean Sharp River sand is obtained and thoroughly washed; gravels are also obtained and washed. Two clean containers are used for the construction of the sand filter that is used in some parts of Africa. The container for the filter and storage could be made out of metal, plastic or traditional clay. A hole is made two-thirds of the way up the filter container and a back flush hose with blocked end and perforated will be fixed at this opening into the drum. The gravel is then placed over it to a height of 7.5cm and the sand is placed above it to a height just below the hose opening. The filter is then thoroughly flushed out with clean water for a week to allow the skin to form. Then constant topping with raw water is done which penetrates through the sand and gravel grains and at the bottom the filtered water infiltrates into the perforations made in the hose which moves through the hose due to pressure and is collected outside through another hose connected from the filter to the storage drum. Both the filter and the storage drum are always covered and the rate of filtration can always be adjusted. There are two types of sand filter systems; Slow and Rapid Sand filter system. But the system would require the biological layer to be formed (bio film or Schmutzdecke) and as such clean water can only be ready after a week. Water filtration is based on the type of granular medium used and the hydraulic arrangement provided to pass water through the medium. The types of Filters are: Slow Sand filters, rapid sand filters,
precoat filters, and up flow filter (Kebreab, 2004). Slow sand filter is made up of sand supported with about 0.5m of gravel with particle size 0.2 to 0.35mm. Water is applied to the filter at a rate of 0.034 to 0.102 l/m$^2$/sec while Rapid sand filters are made up of sand supported about 0.4 to 0.7m layer sand and gravel of 0.3 to 0.6m. Effective particle size is 0.4 to 0.8mm larger than in slow sand and flow rate is between $1 - 2$ and $2 - 7$ l/m$^2$/sec for slow and rapid filters respectively (Wegelin et al, 1991).

### 2.3.2 The advantages and defects of the slow sand filter

In a slow sand filter, the water percolates slowly through a layer of sand bed. During this passage impurities are removed from the water due to a combination of processes. Particles present in the raw water settle on the sand surface. After one or two days these particles form a thin layer on top of the sand bed. This layer, which is also called the filter skin or biofilm (Schmutzdecke), retains even very small particles and also micro-organisms present in the raw water. As long as the water continues to flow, these microorganisms...
remain alive in the filter skin. But the adoption of the biocoagulant sand filter would certainly alter the biological layer.

These microorganisms are the most important elements of the slow filter because they consume the disease causing organisms and other impurities. During the ripening processes, the microorganisms multiply and after sometime they predate on almost all disease-causing organisms and virtually none will pass through the biological skin. This ripening process may take up to a week for new filters and only a few days for filters that have already been used.

However, the filtration rate has to be kept constant in order to maintain its efficacy in filtering up micro-organisms. The materials commonly used locally in Cameroon for the construction of a sand filter unit are as follows: 2 strong plastic or metal drums, 1 ½ yards of hose, four clips, three nipples, strainer or sieve, sharp river sand of 2 different sizes, coal and gravel (Yongabi, 2009). The dimension of the sand is as follows: fine sand between 0.15mm-0.30mm, coarse sand greater than 0.30mm, gravel between 20-50mm and coal between 20-50mm.

2.3.3 Basic filter Materials and Rationale for use

1. Sand as a filter medium is capable of removing suspended solids and it creates an environment for bacteria that can remove organic and inorganic matters like Iron (Fe), Carbon (C) Aluminium (Al) etc., and even rice haul ash from contaminated water. (Barnes and Mampitiyara, 1983)

Gravels: Gravels have been used as filter packs in industrial plants etc., for pre-treatment of water in the form of sedimentation, hence making it very suitable as a filter medium.

Coal: The coal used is in the form of activated carbon produced by subjecting coal to high temperatures in the presence of oxidising gases. Coal becomes very porous after firing giving it a wide absorptive surface for filtration. Activated carbon is used to remove unionised species of metals such as arsenic and antimony which are harmful to the body. It also removes colour, taste and odour from raw water. Activated carbon is also used in polished treatment of drinking water and very pure industrial process water. Extensive research to demonstrate the efficiency of the slow sand filter technology has been carried out (Li et al., 1966; Reid, 1982; Garry et al., 2002; Yarahmadi et al., 2009). The result of these researches demonstrates the efficacy of the Slow Sand Filter in the removal of particles and cysts from turbid water. There have been some modifications of the slow sand filter system such as application of roughing filters for turbidity removal from raw water before filtration with sand filter bed. Additionally, the use of ozone to break down complex natural organic
matter, use of granulated activated carbon (GAC) layer to absorb organics are some recent innovations to improve upon the efficiency of the slow sand filter. However, these pre-treatments may be expensive to apply in developing countries (Muyibi et al., 2002a). The application of natural plant coagulants and disinfectants could provide a cheaper alternative pre-treatment.

2.3.4 Candle Filter (porous ceramics), its setbacks

This is a type of filter sold under a trade name porostone filter which is a ceramic structure having a thickness of 1.5cm through which the raw water must pass through for filtration. The direction of flow is from the outside of the tube to the inside. The removed solids that collect on the outside wall of the tube which can be removed and cleaned before fitting back. The passage of water through the mass of collected solids results in finer degree of separation than would be normal for the porosity of the tube.

Of all the filtration systems, the candle filter is the most easily managed and dependable. The merits and demerits are outlined thus: The most important merit is that it reduces the occurrence of water borne diseases. It provides water that is free of turbidity, colour, odour and objectionable taste. It is easy to manage. The materials for its construction are locally available. Water has to be boiled, thus extra energy with frequent clogging of filter. The system is expensive for rural people and so found only among the affluent in Africa (Pritchard et al., 2009).

2.4 Coagulants in Domestic Water Treatment

A coagulant is a chemical which in solution furnishes ionic charges opposite to those of the colloidal turbid particles in water. Coagulants neutralise repelling charges on the colloidal particles and produces a jelly-like spongy mass called a floc. Flocculation causes considerable increase in the size and density of coagulated particles resulting in a faster rate of settling of the particles in a solution or in the turbid water (Ellis, 1988). Since 1867, lime and slate of ion were applied in reducing the time required for solids to settle naturally (Folkard and Sutherland, 1986). Their use, however, produces high moisture sludge, which requires expensive dewatering equipment. Alum, a metallic coagulant, has been a suitable coagulant in water and wastewater treatment for many years now. It has been, and is still, in use in the form of powder dispensed by one of the several forms of mechanical dry feeder units. This unit automatically controls the amount of alum fed and measures the time of coagulation as well. The early treatment plant added lime directly to water flow; this was inefficient as it did not assist mixing. Today, the dry alum bag is dropped into a solution, which is then transported to the mixing and flocculation basin of the plant (Jahn, 1981).
There also exists polymeric polyelectrolyte coagulants which are long chain, high molecular weight molecules which bear a large number of charged molecules with their net charge positive, negative or neutral. The chemical groups on the cationic polymer are thought to combine with active sides of colloids; such interaction of a single molecule with a large number of particles produces a bridging effect, binding them together into a large particle which settles under the action of gravity. Polyelectrolyte has advantages over metallic coagulants in acting as coagulants; their role as coagulants is similar to that of activated silica. Activated silica is a preparation of colloidal sodium silicate that acts as a coagulant and a coagulant aid in association with alum as observed by Jahn (1984), Kaggwa et al (2001) and Kebreab (2004). Chlorine is the most commonly used synthetic disinfectant in water and wastewater treatment. Very few disinfectants of plant origin are reported commercially.

The use of plant materials to clear turbidity of water is not a new idea. Seeds of Strychnos potatorum tree were used several years back to clear turbid water (Jahn, 1986). Sap from tuna cactus has also been used to clear turbid waters. An aquatic plant commonly known as totora in Peru and Bolivia is used to remove phosphorus and nitrogen from wastewater. However, of all plant materials studied over the years seeds of Moringa oleifera plant have been shown to be most effective as a primary coagulant in water treatment (Jahn, 1986).

For several years indigenous people have relied on their indigenous knowledge and heritage for survival. Historically, there is evidence to suggest that communities in the developing world have used plant based materials as a strategy for purifying drinking water (Miller et al, 2008). Unfortunately, as it always turn out, adequate investigation into indigenous knowledge system in order to validate and improve upon such knowledge is really lacking. There is a need to probe into indigenous knowledge in water purification in order to use that as a springboard for appropriate technology.

2.4.1 A survey of Indigenous Plant materials in Water purification

Corn silk, palm fibres as well as banana or plantain stem bark fibres have been used in many indigenous communities in Cameroon as well as amongst the Igbo’s in Eastern Nigeria for various local filtration purposes. Amongst the palm wine dealers in the North West and South West Region of Cameroon, the use of these fibres to filter turbid palm wine before sale is a common practice. Similarly, these fibres have also been employed for filtration of turbid water when there is no sackcloth in most parts of rural Cameroon, particularly in the olden days (Yongabi, 2009). This trend is similar in Eastern and Western parts of Nigeria and has not been validated. The beauty of this knowledge is to provide a platform for
screening new biomaterials for water purification. It is worth noting that as much as some communities still remember and practice their traditional knowledge, especially in water management, many still do not have or might have forgotten these old methods which are now abandoned and lost. Unfortunately, researchers have not been able to visit and validate this practice in order to develop a resilient and sustainable water treatment systems for these communities.

Furthermore, some tribes in northern Nigeria have used seeds of *Parkia biglobossa* as a Phytodisinfectant for a long time (Sofowora, 1984). The seeds of the plant are pulverised and added to very turbid water and allowed to stand for an hour. The supernatant is then filtered using sackcloth and the water is then boiled for an hour (for long-term storage). In rural areas, the water is then stored in clay pots raised on a sand pile at home (this keeps the water cool). In a survey by Yongabi (2009) respondents strongly acknowledged the use of plants in treating water.

In 1937, Dalziel reported the coagulative and disinfective property of locus bean seeds. The plant (tree) itself has a number of medicinal uses, such as in the management of diabetes with the leaves. The seeds have been fermented locally to produce a local sweetener/condiment for soup recipes (Burkill, 1984a). Another plant used in the past for treating water is Physic nut seeds (*Jatropha curcas*).

The seed powder of physic nut (*Jatropha curcas*) is very useful in wastewater treatment. This plant belongs to the family Euphorbiaceae. Reports on the potentials of this plant in wastewater treatment exist (Yongabi, K.A., 2004). Continuous studies show a very high coagulation potential as well as disinfection. The latex from the leaves equally poses some coagulation activity on turbid water apart from serving as a haemostat (stops clotting). The latex has been used in the treatment of scabies and lice in man (Yongabi, 2004 and Yongabi, 2009). Activated carbon from the husk adsorbs heavy metals from contaminated water. Generally, the seed biomass is made up of about 35% oils rich in cosmetic value and equally used as lubricant oils for engines. The roots of the plant hold the soil intact and, therefore, it is good planted around areas subject to erosion (Shabon et al 2005). Similarly garri, a fermented product of cassava and a common food widely eaten and cherished across Africa, especially Southern and Eastern part of Nigeria and Cameroon, has been used to clarify water. They sprinkle garri in streams/brooks/rivers and after a couple of minutes the water becomes clear for them to fetch (Yongabi, 2009). The coagulation effect may be due to starch (Okafor and Ejiofor, 1986).

Lime/Lemon (*Citrus aurantifolia*) juice has been used as a disinfectant in water since antiquity. It has been squeezed directly into a bucket of turbid water for purification. A
number of communities adopted and used this in the past to treat dirty water, apart from making dirty water fairly clear, it has a greater disinfective property. In some communities, two small lime fruits are added to a bucket of water. Limes are acidic and thus toxic to a range of microorganisms. The fruit juice as well as the rind has shown inhibition on *Escherichia coli* isolates, *Staphylococcus aureus*, *Bacillus* sp. and *Proteus mirabilis* isolated from a range of specimens, including highly turbid water (Yongabi, 2009). This study and the traditional practice are in line with earlier reports of Dalsgaard and Reinchert (1997). The acidity in lime juice is responsible for its disinfective property. Adding lime juice to water (1 – 5% final concentration) to lower the pH bringing it to 4.5 will reduce *Vibrio cholerae* by 99.99% in about 120 minutes. This implies that a pH of less than 4.5 and a treatment time (contact time) of 120 minutes is key to reducing *Vibrio cholerae*. This would, probably, have the same effect on other microbes in water and thus ensure a good quality of drinking water. The pH of water can be lowered with lime/lemon juice to kill the microbes and the pH can be raised using *Moringa* seed powder to neutral (7.0) for drinking. Lime is more acidic than lemon. *Citrus* spp. possess higher disinfectant ability in water than coagulation activity (Dalsgaard and Reinchert, 1997). Another common plant with disinfection activity is *Aloe vera*, also known as *Aloe barbadensis*. *Aloe vera* belongs to the family Liliaceae. The gel of *Aloe vera* possesses various biological and physiological activities such as healing skin burns, prophylactic effect against radiation, leucopoenia, anti-ulcer inhibitory action against a range of microorganisms. There are many folk uses of *Aloe vera* documented by Morton (1961), Babatunde and Yongabi (2008) and K.A. Yongabi (2008) but no documented studies on its biocoagulant activity.

Some previous studies have screened a number of plants as disinfectants for water treatment, *Acrorus calamus* linn (buch) (araceae) Roots, *Anaphalis Cunefolia* Hook (Compositae) Entire plant, *Arnebia nobils* Rachanger (Ratangot) (Boraginaceae) Root, *Eclipta aibba* (linn) Hassk (Bhgangra) , *Hypericum spp.* (Gut ifera) whole plant, *Azadirachta indica* L juss leaf (meliaceae), *Moringa oleifera* Moringaceae fruits, roots, bark, wood stem (Jahn, 1981). Native plants have traditionally been used to improve quality of water in many countries in Africa and Latin America viz. Seeds of *Moringa* used in Guatemala, peach and bean seeds are used in Bolivia as coagulant aids clarify water. It has been reported that dried beans (*Vicia fave*) and peach seeds (*Percica vulgaris*) have been used in Bolivia and other countries of water treatment. Similarly, *Schoenoplectus totora*, an aquatic plant, has been used in Bolivia and Peru for Water Quality treatment (Kebrag, 2004; Miller et al, 2008). *Schoenoplectus totora*, like cattail, is used to remove phosphorus and nitrogen before being discharged to natural drainage systems. The use of aquaculture as a means of treating waste
Chapter 2

water took centre stage and this involves both natural and artificial wetlands as well as the production of algae, higher plants (submerged and emerge), vertebrates and fish to remove contaminants such as Manganese, Chromium, Copper, zinc and lead from water. Similarly, water hyacinth (*Eichhornia crassipes*) has been widely used for the treatment of wastewater, amongst other plants like duck weed, seaweed and alligator weed (Kranert and Hillebrechth, 2001; Shaban et al 2005 and Shuaibu and Yongabi, 2005). Progress continues in the area through invitro experiments employing hydroponics, cultivation of grasses using domestic wastewater is promising as it removes organic matter and suspended solids through physical means, adsorption, absorption and other mechanisms. But in Africa there technologies are yet to yield dividends and may not be possible in the foreseeable future.

A number of seed extracts have been known to flocculate particles. Resin water and the following procedure have been used according to Jahn (1981) and which if developed may yield dividends. It includes; Extract the seeds from the plant/fruit, Dry seeds for up to three days, Grind the seeds to a fine powder, Prepare a mixture of water and ground seed material (the volume of water depend on the type of seed material used) in case of *Moringa oleifera*, add 100cm$^3$ of water for each seed; for peach or bean seeds add 1l of water to each 0.3 to 0.5g of ground material. Mix this solution for 5 to 10 minutes; the faster it is stirred, the less time is required. Finally, after the sediments settle, decant the treated water, testing it for pH, colour and turbidity. But with wetland system; water hyacinth is the most popular plant used in phytoremediation. The water hyacinth, which is a native of South America, is widespread in all the Continents. It thrives well in nitrogen rich environment, and consequently does extremely well in raw and partially treated wastewater. In this regard, wastewater is passed through a water-hyacinth-covered basin, where the plants remove nutrients, suspended solids heavy metals and other contaminants.

2.5 The historical use of *Moringa oleifera* plant in water treatment rural Africa

The seed powder *Moringa oleifera, Lam* has been used in many African societies for water clarification for a long time. Tribes in Northern Nigeria, Northern Cameroon, Chad, Niger, Sudan, Malawi, Ethiopia, Eritrea, Mauritania etc. have a history of use in clarification of turbid water for domestic use (Sutherland *et al*, 1990; Lowell, 2001 and Kebreab, 2004). In some parts of Northern Nigeria in the early days some indigenous people walk with crushed seeds of *Moringa* when going to the farm, and would use it to clarify any suspicious water they came across before drinking, especially on farms that are far away from homes with difficulty of getting potable water. There are a number of publications on the medicinal
potential of the *Moringa* tree as a whole, while there are web resources on the coagulative potentials of the plant as well (Jahn, 1984, Folkard et al, 1990; Folkard et al; 1996; Folkard et al 2000; Kebreab, 2004 and Yongabi, K.A, 2004). A tour of Benue State/ Middle belt of Nigeria in 2005 revealed that many houses/compounds do have at least one *Moringa* tree. It appears there are healthier and larger *Moringa* trees in the middle belt of Nigeria than in the Northern part of the country. Most people in the middle belt, particularly, are aware of its water purification potential, yet it was found that some people consumed dirty (stream) water, and children and women have to travel far distances to hunt for water. It was observed that on the average, a *Moringa* tree in the middle belt in February/March 2005 has 80 pods with an averages of 15 seeds per pod, if one seed treats a litre of turbid water, it means 15 seeds treat 15 litres of turbid water and this is for a situation where the turbidity is high, otherwise half a seed would suffice for mildly turbid water (Personal Communication, 2005). The trees are taller and appear to thrive well in warm and humid environment. The use of the seeds as a soup delicacy is most popular in Benue State. The tree is called Jegede in Tiv Language and Jegene in Idoma dialect, while the Hausas call it Zogale; these are the dominant tribes in Benue State of Nigeria.

### 2.5.1 Seeds of *Moringa Oleifera*

The seeds of *Moringa oleifera* tree have been found to be of great importance and most widely studied. Oil extracted from the seed is used to treat goitre and acute rheumatism and also applied as remedy for hysteria scurvy (Burkill 1985a). The oil is also used in cosmetics production and as lubricants in delicate machines like watches (Ramachandra, et al 1980). The seed is, today, used in water treatment as a coagulant and disinfectant (Eilert, et al 1980, 1981).

The seed contains fixed oils, fatty acids such as palmitic acids, oleic acids, behinic acids, stearic acids and pterygospermin, an unstable substance with low melting point which decomposes readily to benzylisothionate (Ramachandra, et al 1980).
The active ingredient responsible for coagulation, a polyelectrolyte, was isolated in the laboratory and found to be in less concentration during the wet season than in the dry season (Kurap, 1954b and Saluja et al 1987). The seed has been found to have antibacterial activity against both gram positive and gram-negative bacteria (Kurup, et al 1954a). The seeds were found to have antimicrobial effect against all *Staphylococcus aureus*, *Pseudomonas aeruginosa* isolates in vitro while on filter disc inhibited the growth of *Staphylococcus aureus*, *Bacillus subtilis* but not *Pseudomonas aeruginosa* and *Escherichia coli*. It also had activity against the following organisms at certain dilutions: *Bacillus subtilis* and *Staphylococcus aureus* 1: 70,000, *Mycobacterium phlei*, *Salmonella slicotmicellei*, *Salmonella typhi* and *Shigella flexxnei* 1: 40,000 (Jahn, 1986).

The antibacterial action of pterygospermin has been ascribed to one of its molecular components released when pterygospermin breaks down (Jahn, 1986). Pterygospermin has also been reported to have high activity against moulds and fungi (Gopalakrishna, et al 1994). Since the early 1970s a number of studies have been carried out to determine the effectiveness of the seed in water treatment. Laboratory investigations confirm the seed to be highly effective in the removal of suspended solids (Berger et al 1984). Today the seeds of *Moringa* are being used in Guatemala, the Nile region and generally in Africa to treat...
Moringa pterygosperma, a synonym of Moringa oleifera lam, is a deciduous plant with smooth or cocky bark. It can grow up to 8 metres in height and 60 cm in girth. The leaves are imparipinnate compound leaves with up to six pairs of pinnae. Each pinna has opposite pairs of branched pinnules with different numbers and size of leaflets. Between each pair of pinnules is a rod-like gland on the upper surface which easily falls off.

The leaflets are oblong or obviate, the largest about 2.5 x 1.2 cm, which is often oblique with dull green on both sides, and lighter coloured beneath. The plant produces fragrant scented flowers, which are bisexual (Sofowora, 1984). There are five pale green hairy sepals about 1.25 cm long with fine white cream petals, which are unequal and a little longer than the sepals with slender style. The capsules of the fruits are pendulous, linear, acuminate obtusely frigorous and ribbed. The capsule is usually 20 – 45 cm long but sometimes up to 120 cm long. It contains numerous globular seeds about 1 cm wide with three membranous wings at the base and apex (Sofowora, 1984). Moringa oleifera lam is variously called in a number of languages; Moringa oleifera lam or Moringa pterygosperma in English, Horse radish tree, Drumstick, Ben tree, Radish tree, Mother’s best friend, west Indian ben (Lowell, 2001) while in French, Bèn ailé, Benzolive and in Italian; Sandalo ceruleo (Lowell, 2001). It has varying appellations in a range of languages across Africa as well; for instance in Republic of Benin, the Yoruba call it Ewé ilé, while the Bariba call it Yurie ara, Yorwata, yoroguma. As a pan tropical plant its availability across most African countries can be exemplified in the local names. In Yoruba it is called Ewé ilé, Bariba in Benin call it Yuruara, yorwata, yoroquma, while in Fulfulde it is called Guiligandja, naa-nko in Toupouri. In Chad, the Sara clan call it kag n’dongue, while in Ghana it is called yeva-ti (by the Ewe). In Kenya, the Swahilis call it Nkimbo onlonge and in Malawi, the Yao call it Kalokola, in Hausas, zogala, zogall, zogale, Ibo; ikwe oyibo; Fulani; Gawara.
As a globally widespread plant, in Colombia it is called Angela, while in Italian; sandalo ceruleo, of course in English; Horse radish, Radish tree, Drumstick tree, Mother’s best friend, West Indian ben (Lowell 2001) *Moringa oleifera* is naturally a native of India and in Bengali it is called sasna, and in Hindi; Shajmali.

### 2.5.2 Botanic Description / Detail Anatomy of *Moringa Oleifera*

It is a deciduous tree 8m in height and 60cm girth, it possess a smooth and corky bark. The leaves are compound, imparipinnate and up to six pairs of pinnae. Each pinnae has opposite pairs of branched pinnules with different number and size of leaflets. Between each pair of pinnules is a rod-like gland on the upper surface which easily falls off. The leaflets are oblong or obviate, with the largest about 2.5 x 1.2cm. The leaf base may be variously described as acute, obtuse or rounded and is often oblique. The apex could be described as obtuse, rounded, or emargenate, entire, dull green on both sides and lighter coloured beneath. Generally, the plant produces fragrant scented flowers, which are bisexual; the flowers are in a loose auxiliary panicle which is up to 15cm long. Apparently, the flower stalks are up to 1.25cm long and very slender with 5 pale green hairy sepals about 1.25cm long with like white-cream petals. These petals, apart from being unequal are a bit longer than the sepals, and thus possess slender style. The capsules of the fruit are pendulous, linear, acuminate, and obtusely trigonous and ribbed capsule which is usually 20 – 45cm long but sometimes up to 120cm long. The tree produces seedpods with numerous globular seeds about a cm wide with 3 membranous wings at the base and apex (Ramachandran et al., 1980).

The leaf juice possess anti helminthic activity while the seed oil is used for treatment of goitre, rheumatism, listeria and scurvy. The tree gum (tragacanth – like gum reddish in colour) is used in the treatment of diarrhoea, ante febrile, as a diuretic and for asthma. The gum is made of resins. The root bark is an anti-irritant, while the whole roots are used in the treatment of epilepsy, nervous debility, cardiac and circulatory problems, fever, leprosy and mouth sores.

The seed oil has a wide application in cosmetics and can absorb and retain odour. The oil can serve as lubricant for delicate machines and watches. The pounded leaves are used as sponge for scrubbing utensils and walls. In Benue state of Nigeria, *Moringa* seeds (amongst the Tiv and Idomas) are ground and the powder mixed with water in a reservoir and allowed to stand. The suspended solids coagulate and settle to the bottom resulting in a clear reservoir of water, then decanted and filtered for household uses. The *Moringa* vegetable is a delicacy in soup and many household tables and with so many positive health attributes. The challenge is to come up with appropriate biotechnology and bioengineering applications.
for wider use. *M. Oleifera* Lam (synonym: *M. pterygosperma* Guertner) is a native of Agra East India, South of Himalaya Mountains. It belongs to the family Moringaceae, a flowering plant. Its characteristic pinnate leaves couple with DNA analysis show close relationship with the Caricaceae family where *Carica papaya* (Pawpaw) belongs. There are 13 species in this family; *Moringa oleifera* lam (pan tropical), origin India, *Moringa peregrine* (origin Arabia and Red Sea area) and *Moringa concanensis* (origin India, Pakistan and Bangladesh). Others include *Moringa drouhardii*, *M. ovalifolia*, *M. hildbrandtii*, *M. Stenopetala*, *M. arborea*, *M. bornziana*, *M. longituba*, *M. pygmaea*, *M. rivae* and *M. ruspoliana*. *Moringa oleifera* leaves and seeds possess a broad range of chemicals; proteins, fat, carbohydrate, fibre, minerals, calcium, copper, iron, potassium, magnesium, phosphorus, sulphur and selenium. Other coagulants in Peru, especially mucilaginous sap from Tuna leaves obtained from certain species of cacti have been used in treating water. Similarly, seeds of numali tree (*Strychnos potatorum*) has been used to clarify river water dating some 4000 years ago (Sanskrit writings) (Burkill 1985b). Strong evidence exist to show that some of these plants especially *Moringa oleifera*, compares favourably with Alum in turbidity reduction in raw water (Jahn, 1979)

### 2.5.3 Chemical Constituents of *Moringa oleifera* (lam)

*Moringa oleifera* Lam contains several phytochemicals, some of which are of high interest because of their medicinal value. In particular, *Moringa oleifera* or the Moringaceae family is rich in a fairly unique group of glycoside compounds called glucosinolates and isothiocyanates. The effectiveness of the *Moringa* plant in treating ovarian cancer has been linked to the ability of benzyl isothiocyanate (BITC) and phenethyl isothiocyanate (PEITC) to induce apoptosis in ovarian cancer cells in vitro (Kalkunte et al, 2006; Satyan et al, 2006). There is even evidence supporting the antitumor activity of isothiocyanates in cancers of the lung, breast, skin, oesophagus and pancreas. *Moringa oleifera* leaves contains 2 nitrile glycosides, naizirin and niazirinin, and 3 Mustard oil glycosides, 4 [c4’ – O – acyl-alpha – L – rhamnosyloxy benzyl] Isothiocyanate, niaziminin A and B which are reported to have hypotensive activity. Besides, beta-sitosterol, glycerol-1-(9-Octa decanoate), 3, - 0 – (6’ – 0 – oleoyl – beta – D – glucopyranosy) – beta –sitosterol and beta – sitosterol – 3 – 0 – beta – D glucopyranoside have been identified. The root bark of *Moringa oleifera* contains two alkaloids; total alkaloids 0.1%, which are Moringine known to be identical to benylamine and Moringinine known to belong to the sympathomimetic group of bases. Other phytocomstituents in very small amounts include; essential oil with a pungent smell and phytosterol while waxes and resins are found in the entire plant. Furthermore, a rich and rare combination of zeatin, quercetin, beta-sitosterol, caffeoy/quinic acid, pterygospermin and
kaempferol has been identified in the plant as well. These components are also found in other *Moringa* species but in varying quantities, but studies are still inadequate on the other species (Munyaziza and Yongabi, 2007).

### 2.5.4 *Moringa oleifera* dosing in water treatment

The use of *M. oleifera* seeds in water treatment is well known in rural Africa particularly in Malawi, Mauritania, Sudan, Northern Nigeria, etc. There is no harmonized method of using *Moringa* in water treatment in the rural communities. For instance in Northern Nigeria, a seed is used to treat 1 litre of water while other locals used two seeds for the same quantity of water. However, Lowell, (2001) describes a, somehow, standard method for water treatment with *Moringa*: The seeds (mature seeds are brown) are shelled, the seed kernel is then crushed, (using dry head blender) and sieved using a 0.8 mm mesh (maize flour sieve is OK). Two teaspoons of the powder (2g) is mixed with a bit of clean water to make a paste and mixed in 20 litres of turbid water by stirring. Lowell (2001) and Kebreab (2004) recommend one seed per litre of turbid water and one seed per 2 litre of fairly turbid water. The author recommended that the paste has to be given five minutes so that the coagulant protein extracts in water, before use. The insoluble materials are removed by filtering through muslin (cloth or fine mesh screen) into the bucket of water to be treated. The water is then stirred rapidly for two minutes, slowly for 10-45 minutes and the setup is then left undisturbed for 1-2 hours. It is then decanted carefully. The water is then boiled or filtered off and bacteria killing substances such as chlorine (bleach) 1 to 2 drops per litre is now added to the water to make it completely safe for drinking.

This method would certainly generate safe drinking water but the last treatment procedure where chlorine or bleach is used may raise a number of questions as to its environmental friendliness and secondly the likely hazards that may be inflicted upon the users who are likely to be rural and uneducated people. As a matter of fact this method is limited and there is a need to come up with a completely chemical free means of treating water without the use of energy. Most studies on *Moringa oleifera* has been focused on small scale household level which is very beneficial (Jahn, 1981 Folkard et al 2000) but household recontamination of water still remains a problem for rural Africans. Focus has been placed on the isolation of active ingredients (as water treated with *Moringa oleifera* seeds gets recontaminated after 48hours) as well as the fact that the seeds loose its coagulant property in a year. The option of synthesis and expensive isolation procedure (Kebreab, 2004) may be time consuming and expensive compared to other methods. Previous studies have actually acknowledged how indigenous people in Africa and elsewhere in the world treat their water. Jahn (1979) reported that in Africa, particularly Chad, Sudan, Tunisia and
Nigeria, indigenous plants were added to drinking water by rural villagers to remove turbidity, unpleasant tastes and odours.

*Moringa oleifera* seeds have a storage life of about one year and three months, depending on the geographic/climatic region as well as conditions of storage. However, it seems there are some biocoagulants with a longer shelf-life on bench than *Moringa oleifera*, and the effect can be appreciated on the storage time when water is treated with *Moringa* and other biocoagulants. Garri and *Aloe vera* as biocoagulants have a longer shelf life than *Moringa oleifera*. Similarly, starch-based biocoagulants have a longer shelf life than protein-based biocoagulants. There are similarities of cultures across the globe, so some of the traditional methods of water treatment cited herein may be similar in other parts of the world. There is the need to revive these methods and find out how biocoagulant lives can be improved. The problem of water quality in rural Africa is chronic and will never be solved in the near future if concerted efforts towards the appraisal and, perhaps, revival of the local technologies are not implemented in an organised manner i.e. looking for cheaper alternatives rather than expensive high technology. Another problem is that even with donor assistance in the development of potable water supply schemes using high technology, most rural areas where people rely on subsistence farming cannot manage or maintain their water sources using such high technology. The dwindling economic condition in most African countries will necessitate alternative technology with local available materials to complement existing conventional water treatment, rather than importing chemicals from abroad at elevated costs, whereas the indigenous ones could be developed Kebreab (2004). The overall financial cost and skills involved may be limiting for wide scale exploitation of advanced technologies in Africa.

*Moringa oleifera* trees have numerous advantages; they are hardy and drought resistant, fast growing and with a large number of seeds. They are a non-toxic and effective coagulant for turbidity and bacteria control. These technologies are traditional, rudimentary and easy to implement, thus ideal for rural areas.
2.6 Conclusion and Recommendation

Water treatment technologies currently used may not provide dependable solutions to the water crises due to cost and high energy inputs, amongst other reasons, and the sand filter has a number of defects with time. There is however a growing body of plant-disinfectants and coagulants that needs to be developed. To add, research work in the last several years has focused extensively on promising coagulants – Moringa oleifera with interest in the isolation of the coagulant protein. A Phytodisinfectant sand filter could provide a cheaper alternative.
CHAPTER 3

METHODOLOGY

This chapter describes the methods applied to document indigenous knowledge in water treatment and to proof the efficacy of a phytodisinfect and sand filter system.

3.1 MICROBIAL AND PHYSICOCHEMICAL ANALYSES OF WATER SAMPLES.

Three water samples were collected from a shallow well or pond, usually consumed untreated, in Bamenda, Cameroon. The samples were tested for the following total aerobic Mesophylic bacterial counts, coliform counts, E. coli counts as well as total fungal counts according to methods of Cheesbrough, (1984) and Yongabi, (2004) and the specific identities of organisms confirmed using keys specified in Bergey’s manual of Determinative Bacteriology (Buchanan and Gibbon, 1980; Cheesbrough, 1984) after gram staining and microscopic studies on the micro- morphologies as well as appropriate biochemical tests.

Figure 14: Bench scale laboratory rig to demonstrate Moringa-sand filtration
(Yongabi, 2010)
Microscopic examination of the water samples to report possible parasites and unicellular organisms were also carried out (Harrigan and McCance, 1976). The mean values of the bacterial counts for three samples were reported and compared with the WHO standards for potable water. These analyses were done before and after treating the water with the plant coagulants collected from the field.

Fungal analysis was carried out. Discrete fungal colonies were observed microscopically in a lactophenol cotton blue preparation. The cell morphologies as well as unique differential features were recorded. The characteristic features collated were compared with taxonomical keys specified in Barnett and Hunter (1972) and Ellis et al., (2007) to identify the fungal isolates. The mean fungal counts before and after treatment with the plant coagulant and disinfectants was recorded. This approach of counting and identifying specific microorganisms from water, rather than just the indicator organisms, was useful in assessing the efficacy of new disinfectants of plant origin, bearing in mind that some organisms do not correlate well with indicator organisms. The pH of the water samples in the field were tested using both a combi-9 test strip (a standard strip for routine urinary biochemical analysis) or pH meter (HACH DR, 2000). The turbidity of the water samples were analyzed using a UV visible spectrophotometer HACH model 44600; each analytical parameter was done in triplicates and mean values recorded.

3.1.1 Plant collection, processing and selection for detail studies

The plants were collected based on a survey of its local uses in water purification by the indigenous people of Cameroon. Previous studies done (Yongabi, 2004) revealed that *Moringa oleifera* is well known and very effective coagulant provoking more focus in this study. *Moringa oleifera* (lam) seeds were collected around homes in northern and north western Cameroon during the dry season, while sclerotium of *Pleurotus tuberregium* frs (singer) was collected from south western part of Cameroon (where it was abundant). *Aloe vera* was collected from the compounds within Bamenda and processed based on the
indigenous information that was gathered. A rapid bench scale screening for coagulation and antimicrobial activity was done (Yongabi, 2010). Based on this, *Moringa oleifera* was selected for further detail studies.

### 3.2 PROCESSING *MORINGA OLEIFERA* PLANT BIOMATERIALS

The seeds of *Moringa oleifera* were dried under shade after seeds had been removed from the pods, and 1000 grams was collected for use in coagulation and extraction studies. The seeds and shells were ground using a kitchen blender to obtain a moderate powder that was used throughout the study (Kebreab, 2005 and Pritchard *et al.*, 2009).

#### 3.2.1 Coagulation studies using *Moringa oleifera* powder on turbid water

Previous jar (beaker) test studies show that one seed of *Moringa* is adequate to purify moderately turbid water of 150NTU (Folkard *et al.*, 2000; Kebreab, 2005 and Pritchard, 2009). In this study, however, graded weights (0.5g to 5g) of each of the plant materials and *Aluminum sulphate* (Alum) was added to 200mls of each of the wastewater samples in 250ml capacity beakers. This was done according to methods of Jahn (1988) and Folkard *et al.* (2000). Two hundred mls (200) each of the water samples was filled in a 250 ml capacity beaker placed on a slot. One gram, two grams and five grams each of *Moringa oleifera* seed powder was added to each of the water samples in a beaker and agitated at 150 rpm for two minutes. The mixing speed was reduced to 30 rpm and kept for 30 minutes residence time. Residual turbidity of *Moringa* solution, water samples as well as the treatments was recorded using a turbidimeter (Ana-148photoelectric model) against a blank. Coagulation was calculated based on Lee’s equation: Coagulation activity = RT blank-RT sample/RT blank (RT is residual turbidity) (Okuda *et al.*, 2001). Each treatment was set up in duplicate and average results recorded. The coagulation (turbidity clearance), disinfection ability (total bacterial counts) and pH were monitored and average of duplicates recorded. Data was collected after 15 minutes, 30 minutes and one hour for all the above parameters, on the same day.
In order to validate part 1, objectives 1 and 2, four experimental rigs were constructed. A series of experiments were carried out to prove the concept that *Moringa*-disinfectant sand filter system could enhance water purification. The four experimental rigs were constructed at the Engineering workshop of Adelaide University according to specifications described by Hatt *et al.*, (2005). The test rigs were made of plastic acrylic tubing of bed depth 0.5m (50cm), with external diameter of 50mm, inside diameter of 44mm and thickness 3mm. An allowance of 2 cm (20mm) was allowed at the bottom and top portions of the rigs. At the bottom, it was packed with porous glass disc or rock wool while at the top space was provided for turbid water to sit. Unlike previous rig designs (Hatt *et al.*, 2005, Pritchard *et al.*, 2009), the test rigs in this research were modified. Four Teflon taps with plastic valves was fitted at a 12.5 cm distance on the walls of the rig. This was done to collect filtered water at various bed depths every 15 minutes residence time for up to an hour. The feed reservoir was a five litre plastic bucket with a collector which filtered water was collected (rig diagram attached as in Figure 15 and Figure 16).
Figure 15: Body filter

Figure 16: Water filter
3.4 Preparation of Synthetic Contaminated Water

An experimental strain of *Escherichia coli* (ATCC11775) isolate from the Institute of Medical and Veterinary Science (IMVS) was collected. A subculture of the *E. coli* was made on MacConkey agar and broth and confirmed using gram staining technique and API-20E bacterial identification test strip. Single colonies of broth cultures of *E. coli* was inoculated into 500mls of nutrient broth and incubated over night at 37°C (Pritchard *et al.*, 2010). Following incubation, the culture was centrifuged at 300rpm for 5minutes. The supernatant was aseptically removed by decanting and the *E. coli* pellets re-suspended in one litre of distilled water (MilliQ water). The centrifugation process was repeated to ensure that all nutrient broth was removed from the *E. coli* pellet. This was further re-suspended in one litre of sterile distilled water (MilliQ water) to give a final wash stock suspension of *E. coli*. The *E. coli* count was taken by plating one ml of the suspension on plate count agar (Oxoid Ltd) in triplicate and the mean *E. coli* count was recorded as colony forming units per ml (Yongabi, 2004; Pritchard *et al.*, 2007 and Pritchard *et al.*, 2008). The works of these previous authors observed that up to 30, 000 faecal coliform per 100ml were present in some surface water supplies in Nigeria and Malawi. As *E. coli* is considered the most suitable index of faecal contamination (WHO, 2006), a target dose of *E. coli* for the samples that were tested were set at $1 \times 10^4$ to $3 \times 10^4$ colony forming units per 100ml.

3.4.1 Preparation of *Aeromonas hydrophila* for Disinfection studies

An experimental strain of *A. hydrophila* (wild strain) isolate from water was collected from IMVS, Adelaide. The choice of selection of this organism is based on its growing resistance to chlorination (Yongabi, 2004). Furthermore, indicator organisms such as *E. coli* and faecal streptococci do not correlate well with specific groups of organisms such as *Aeromonas spp.* in water ways. A subculture of *Aeromonas* was made on nutrient agar (Oxoid Ltd) and nutrient broth (Cheesbrough, 1984) and confirmed using the API-20E
bacterial identification test strip at IMVS. Single colonies of broth culture of *A. hydrophila* was inoculated into 500ml of nutrient broth and incubated over night at 37°C (Pritchard *et al*., 2010). After incubation, the culture was centrifuged at 300rpm for 5 minutes (Pritchard *et al*., 2010). The supernatant was aseptically removed by decanting. The *A. hydrophila* pellets were resuspended in one litre of sterile distilled water (MilliQ). The centrifugation process was repeated to ensure that all nutrient broth was removed from the *A. hydrophila* pellet. From the washed stock suspension, the *A. hydrophila* count was enumerated by plating one ml on plate count agar (Oxoid Ltd) in triplicate and the mean counts recorded as colony forming units per ml. An approximate dose (infective propagule) of 1000 colony forming units per ml of *A. hydrophila* for the samples was standardized in the range of 1 x 10^4 to 3 x 10^4 colony forming units per 100ml.

### 3.4.2 Preparation of Synthetic Contaminated water using Soil

To study total heterotrophic bacterial Counts for Disinfection studies with the test rigs fifty (50) grams of humus soil was collected from the rhizosphere of a flower bed, suspended in one litre of sterile distilled water and agitated for five minutes on a shaker. The turbidity was taken for two samples using a turbidometer. The pH of the same two samples was read off using a pH meter. The total heterotrophic bacterial count per gram of soil as well as total fungal counts was determined by culturing on nutrient and potato dextrose agars respectively. Each set of cultures were incubated at 37°C for 24 hours and at room temperature for yeast growth (Pritchard *et al*., 2007 and Pritchard *et al*., 2010).

### 3.4.3 Preparation of turbid water using bentonite for Coagulation studies

Beaker or Jar test studies according to Kebreab (2005) were applied. The methods used earlier by Kebreab (2005) and Pritchard *et al*., (2009) were slightly modified. Turbid water was made using a hundred grams of bentonite added on to a liter of sterile distilled water. This was stirred for three minutes and allowed to stand on a shaker for 24 hours for complete hydration. Desired turbidity was obtained by dilution. A turbidity of 150NTU to
Chapter 3

500NTU was used for coagulation studies. This turbidity value reflects the average turbidity of surface water from Bambui and Mile 6 Mankon, Cameroon. The Optical density at 500nm using a UV spectrophotometer or a turbidometer was taken against a water control. The pH of the turbid water was recorded using a calibrated pH meter.

3.4.4 Preparation of synthetic hybrid turbid and contaminated water

A hundred grams of bentonite was spiked onto a litre of sterile tap water to generate a turbidity in the range of 100 to 500NTU. One gram of soil was added on to it. This was agitated for 3 minutes. Five mls of *E. coli* and *Aeromonas hydrophila* containing at least 1000 colonies from the stock suspension was added and mixed carefully in a 50 ml vortex tube to disperse the bacteria within the bentonite mixture.

3.4.5 Preparation of model hybrid turbid water with bentonite and soil.

A hundred (100)grams of bentonite was added onto a litre of sterile distilled water (MilliQ water) containing 50 grams of soil previously used for the analysis of mesophilic bacterial and fungal counts. The suspension was carefully mixed to disperse the soil and the bentonite. The turbidity was taken and adjusted where appropriate to fall between 100 to 500 NTU. The pH was taken using a pH meter. The total heterotrophic bacterial and fungal counts were taken by culturing on nutrient and potato dextrose agars respectively for 24 hours at 37°C (Kebreab, 2005).

3.4.6 Data collection frequency from the rig experiments.

A pre-treatment using 200ml of each of the synthetic water samples prepared above was carried out in beakers using 0.5 and 1 gram of *Moringa* seed powder (Muyibi et al., 2001) at a residence time of 15 and 30 minutes. This was done in duplicate, the pH, turbidity, *E. coli*, *Aeromonas*; total bacterial and fungal counts were taken in duplicate for each of the *Moringa* concentrations. The same water samples were run through the sand filter bed (Particle distribution size of the sand is 0.1mm to 1.5mm), packed at a bed depth of 0.5m. Two samples of the filtered water at every 12.5cm bed depth for four bed depths...
was taken at residence times of 15 minutes, 30 minutes and one hour. Results were recorded in triplicate.

Two synthetic water samples were run through the sand filter rig with a 2mm bed of *Moringa* seed powder placed on top. Two filtered water samples at 12.5 cm interval depths to 0.5 m depth were taken for the same analyses as per above. The flow rates were determined by measuring the number of drops of water per minute and the volume of filtered water per minute was determined using a measuring cylinder.

The frequency of backwashing of the filter was determined by the clogging rate and level. However, after each set of experiments, the sand was autoclaved and washed thoroughly before reuse. Data collected was analyzed using appropriate statistical tools and presented using graphs and bar charts (Gay, 1992).

3.5 **Solvent Extraction of *Moringa oleifera* (MO) Seeds.**

A cold extraction using solvents of varying polarity in the order: hexane, toluene, acetone, methanol and water on 50 grams of MO seed powder were carried out. 50 grams of the plant materials was steeped in 250ml of solvent (95% purity) in a 1:5 weight per volume ratio for 24 hours (Pavia, 1976; Cannel, 1998). Filtration was done using gravity and solvent was evaporated in a fume cupboard under a gentle stream of nitrogen gas (Balandrin *et al.*, 1985). The nature and yield of the solvent extracts from *Moringa* seeds and *Moringa* seeds plus shells was recorded by using a weighing balance. The purpose of this was to generate an organic extract that was tested on microbial strains isolated from the water sample. Any antibacterial activity in vitro will also support whatever disinfection results were obtained during the bench scale study (Yongabi, 2004). A salt extraction of *Moringa oleifera* seeds was also carried out according to methods of Okuda *et al* (2001a) and Kebreab *et al* (2005). Salt extraction of *Moringa* active coagulant components have been reported as cost effective. Salt extracts were generated in this study in order to
compare its efficacy with polarity solvent extracts. A five percent salt solution was prepared by dissolving 5 grams of salts in 100 mls of MilliQ water. Twenty grams of *Moringa* seed powder was then steeped into 100ml of 5% salt solution for 48 hours. This was filtered by gravity using Whatman filter paper no.13 to generate a salt extract of *Moringa*.

### 3.5.1 In-vitro antibacterial assay

The solvent extract of MO based disinfectants was tested on bacterial strains (*E. coli* ATCC1175) and *Aeromonas hydrophila* indigenous in the water and preserved on nutrient agar slants in the laboratory. The agar diffusion method previously described by Cheesbrough (1984) and Rogo *et al.* (2010) was applied. A serial dilution containing 10,000 colonies of each of the bacterial isolate was incorporated aseptically into molten nutrient agar and allowed to solidify in a sterile inoculating cabinet. Zero point two (0.2) mls of each of the extracts in a concentration 0.05mg per ml was aseptically introduced into the appropriately well labeled wells (British Pharmacopoeia, 1998; Rogo *et al.*, 2010). Wells of 6mm in diameter were bored into the agar using sterile brass steel borers. Clear zones of inhibition around the wells where extracts had been placed were measured using a ruler and recorded as diameter zone of inhibition in millimeter (Ebana *et al.*, 1991; Ntiejumokwu and Alemika, 1999). The setup was done in duplicate and mean values recorded. Two control wells each using the extracting solvents; hexane, toluene, acetone, methanol and water were also used. A synthetic coagulant control (aluminum sulphate) and a synthetic disinfectant control (sodium hypochlorite) were used as controls.

### 3.5.2 Determination of Minimum Inhibitory Concentration (MIC)

The MIC was determined for the active extracts on each of the test organisms. This was done on Aluminum sulphate and sodium hypochlorite if found active against the test organisms. The method of Rogo *et al.* (2010) was applied. The initial extract concentration of 0.05 was further diluted 10 times (0.005mg/ml) and each concentration
tested on the extract. The same concentrations were applied for the controls. The lowest concentration with inhibition was taken as the minimum inhibitory concentration.

3.5.3 Preliminary Phytochemical analysis of *Moringa oleifera* extracts

Phytochemical screening was done of the active extracts. This was carried out in order to elucidate the possible active ingredients present in the seed that may be responsible for coagulant and disinfection activity. The tests for alkaloids, saponins, tannins, flavonoids, steroids, terpenoids, phlobatanins and glycosides were carried out according to methods previously described by Rogo *et al.*, (2010). For Alkaloids, the dragendorf and wagner reagents were added onto 0.2 mls of each of the extracts. The presence of turbid precipitates was indicative of the presence of alkaloids. The froth tests were used for saponins. Heating of 0.2mls of the each extracts in a test tube for 5 minutes with persistent frothing observed was indicative of the presence of saponins.

Tannins were tested by applying the ferric chloride test. Two drops of ferric chloride were added onto 0.2 mls each of the extracts and the formation of a green precipitate after five minutes indicated presence of tannins. Precipitates with appropriate colors after addition of the appropriate reagents onto 0.2 mls each of the extracts indicated the presence of phlobatanins, steroids, flavonoids, terpenoids and cardiac glycosides.

3.5.4 Chromatographic analyses.

Thin layer chromatography was carried out on the active extracts. The extracts were spotted on a thin layer plate coated with silica gel and allowed to stand in a solvent pool. After elusion, the plates were dried in an iodine chamber and the retardation factor calculated for each of the spots observed (Cannel, 1998). A number of solvent mixtures, for example, 100% methanol and water, 100% hexane, 50% methanol and 50% water were used in the solvent pool as a mobile phase. The results of the separation were recorded. Other separation techniques such as infra-red spectroscopy and Proton NMR were carried out on the active extract. This could provide useful information on the nature of the
coagulant and disinfectant ingredient in *Moringa*. This was done in the Badger laboratory in the School of Chemistry, The University of Adelaide.

### 3.6 Construction of a Pilot System

After ascertaining the efficacy of the plant coagulants in turbidity removal through the rig experiments as well as the rapid screening tests to select coagulants a pilot *Moringa*–sand filter hybrid system was constructed at the Phytobiotechnology Research Laboratories in Bamenda, Cameroon. Two systems were constructed; a compact Biocoagulant/disinfectant – sand filter drum for household use with a carrying capacity of 150 liters of water was evaluated using turbid surface water from Bambui and Mile 6 Mankon, Bamenda, Cameroon.

![Figure 17: Location of Cameroon in Africa](image)

One system served as control (only sand filter bed), one tests system (MO-Sand filter bed). The water sample was filtered through the systems, and the microbial, pH, and turbidity assessed before and after filtration. Standard microbiological protocols were used (Cheesbrough, 1984) and potability compared to WHO and APHA standards, while pH and turbidity was analyzed using the HACH (model 44600) DR 2000.
Figure 18: Community participation and interest on the pilot water project
(Yongabi, et al., 2012)
CHAPTER 4

A Moringa oleifera disinfectant sand filter integration. A review of an alternative sustainable technology for household water treatment

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This chapter presents Article 1. The article presents an overview and review of one of the plants – Moringa oleifera – widely used in water treatment for more than three decades. The extent of research carried out on this plant and the level of efforts made in integrating this plant with a slow sand filter to generate water in a relatively shorter retention time was reviewed. This article was published in the Journal of Environmental Science and Engineering in 2011, Vol 5, pp 1100-1108.

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Figure 19: Moringa plant and seed pod (Yongabi et al., 2011)

Yongabi, K.A., Lewis D.M and Harris, P.L (2011). A Moringa oleifera disinfectant sand filter integration. A review of an alternative sustainable technology for household water treatment. Journal of Environmental Science and Engineering, 5, pp 1100-1108. This article is highly cited in Asian and African countries. This journal has a reasonable impact factor and is run in Illinois, USA and in China and available in hard cover as well as online:

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Abstract: This review provides an insight and up-to-date information on the application of *Moringa oleifera* seeds, the shortfalls of existing technologies as a coagulant and disinfectant in domestic water treatment. While the coagulant properties are well reported, the disinfectant properties are not well studied. Literature on low cost alternative technologies such as the application of biocoagulants and slow sand filters are extensively reported. However, there is limited work addressing the limitations of these technologies that have restricted their widespread use to solve the global water crises. Slow sand filters have a very slow filtration rate that depends on the biofilm layer which takes about 17 days to form. *Moringa oleifera* treated water cannot last more than 48 hours without bacteria regrowth. Investigation of the best method of isolating coagulant component continues with differing opinions over the nature of its coagulant ingredient not resolved in ongoing literature. An attempt was made in this paper to highlight the advantages of a *Moringa* disinfectant sand filter hybrid system that can purify water. Microbiological advantages of this system in providing a 100% removal of pathogens, and engineering considerations such as water treatment within an hour residence time, faster flow rates, less clogging and backwashing could be some of the advantages of a *Moringa* sand filter system. The need to focus on integrating *Moringa* and sand filter systems for more practical applications is recommended.

Key words: *Moringa oleifera*, disinfectant, sand filter, water, integration, review.

1. Introduction: An Overview of Research Work on the Use of *Moringa Oleifera* Seeds in Water Treatment

Potable water and adequate sanitation are essential for human health. However, 1.2 billion people do not have access [1]. Furthermore, close to 2.5 billion people lack adequate sanitation facilities [2-5]. The most important step in water purification is disinfection [6-9]. Coagulation, flocculation, sedimentation, filtration and disinfection is used worldwide in the treatment of water [10] and aluminium sulphate and chlorine are by far the most widely used coagulant and disinfectant in water treatment [11-13]. The present well documented technologies used in water treatment such as reverse osmosis, ion exchange, ultraviolet (UV) sterilization, synthetic coagulants and disinfectants are not cost effective [14]. The formation of tetra and trichloromethane by the application of chlorine in water treatment has been reported [15] and chlorine is corrosive to the skin [14]. The application of synthetic disinfectants and coagulants could be hazardous to the environment as it affects non-target organisms in the soil [16], and is generally non biodegradable [1] expensive to run, manage and maintain [14, 16], where synthetic coagulants and disinfectants are imported
into Africa at expensive cost [17]. Aluminum sulphate cost 50 US dollars per kilogram while calcium chloride cost 1000 US dollars per kilogram [18, 19]. On the other hand, reverse osmosis and UV sterilization requires high energy input but most African countries lacking adequate electricity [1, 6]. Low cost technologies such as the slow sand filter are well known. However, the slow sand filter which has been used for than a hundred years in water treatment has a very slow flow rate as it takes more than three weeks for the biofilm filter layer to be formed [20]. The limitations of the conventional water treatments have increased interest in the use of organic coagulants, biocorrosion resists and disinfestants derived from plant materials such as water hyacinth [21], chestnuts and acorns [22]. *Moringa oleifera* seed [1, 17, 23-28]. *Moringa oleifera* seeds have demonstrated large effects on turbidity settling between 92-99% reductions [13, 24]. Extensive studies on the disinfection property of the seeds are lacking. Water treated with Moringa seed extracts produces less sludge volume compared to alum [29], but the main setback in water treatment is the significant increase in organic content [15, 30]. To this effect, Jahn et al. [25] reported that water treated with crude Moringa cannot be stored for more than 24 hours. Therefore, the crude extract of Moringa is generally not suitable for large scale water treatment applications where the hydraulic residence time is high [14, 15, 17]. Research in the last thirty years on *Moringa oleifera* have been directed toward reducing the organic load from the seed extracts through the isolation of the active coagulant protein [14, 15, 17, 24, 26, 31]. Extraction and purification of Moringa active ingredient has provoked interest with most reports attributing its coagulant activity to an antimicrobial protein [14, 31, 32]. However, other reports suggest that the active coagulant ingredient is neither a protein nor a carbohydrate but an organic polyelectrolyte [15]. The various conflicting reports on the nature of coagulant proteins do not seem to address the soaring water crisis in Africa [32]. A low cost technology that integrates *Moringa oleifera* properties and the slow sand filter may provide a sustainable alternative to water treatment. In this study, a review on the factors that may hinder the application of Morinaga seeds in water treatment is highlighted through a critique of available literature. The advocacy that research should be directed toward application of Morinaga-disinfectant-sand filter integration that can generate clean water at household level in a day is emphasized. The future outcome of this paper is to trigger research interest and focus in the practical application of this alternative technology for household water purification in resource limiting countries.

2. Background: An Overview/Review of Water, Hygiene and Poor Environmental Sanitation in Developing Countries

Approximately five million lives are lost annually due to drinking and use of contaminated water [17, 33-35]. Globally, four billion cases of diarrhoea causing 1.8 million deaths are reported every year [36]. Out of this, 90% are children below the age of five [5, 34]. In sub-Saharan Africa, diarrhea morbidity is about 17% [37]. Water scarcity and poor environmental sanitation is increasing. This is mainly due to the disproportionate increase in population, global climatic change, drought cycles, deforestation and desertification particularly in Sub-Saharan Africa [19]. The population of Sub-Saharan Africa as of two decades ago is increasing steadily at an unprecedented rate of 2.6% per annum [38]. In Nigeria, for instance, population increases have been approximately 3.0%, while Ghana and Burkina Faso have growth rate of 2.9% [38]. The trend is similar for many countries in Sub-Saharan Africa and could have doubled in 2010. This implies increasing pressure on water resources [1, 35, 39].

Ground and surface water is the main source of drinking water for more than 70% of the population in Africa [1, 20, 40, 41]. The most common source of drinking water in Africa particularly for Cameroonians is stream, boreholes, shallow wells, springs and rivers.
These water sources are unprotected [8], and usually consumed without any form of treatment [1]. A number of waterborne and water related diseases such as typhoid, paratyphoid, diarrhea, cholera and dracunculosis are fast becoming endemic in most developing countries [44, 45]. These pathogens are commonly derived from human fecal material [34, 39, 46] in the rainy season, many pit latrines in the developing countries collapse under their own weight due to poor construction, further reducing sanitation coverage. Furthermore, open defecation in the bush and in water bodies is still a common means for human excreta disposal for rural populace without access to pit latrines [1]. In the rainy season, fecal matter from pit latrines and open sources is washed into surface water thereby contaminating the water [47]. In Malawi, for example, microbiological water quality from shallow wells (with depths, typically not exceeding 15 m has been found to be more inferior in the wet season compared to the dry season [1, 34, 46]. However, the situation could have easily been eradicated through proper sanitation and provision of adequate clean water [1, 32]. In developing countries, particularly in developing countries, water treatment is quite expensive and the ability to pay for services is very low and low cost technology is scarce. In Cameroon and Nigeria, for example, aluminum sulphate is imported from Europe at a cost of 60 USD per kilogram and calcium chloride cost 45 USD per litre. In most African countries more than 70% of its populace lives below the poverty line [48-50]. Reports for more than three decades indicate strongly that this poverty trend could be reversed if focus is laid on sustainable water treatment systems that are low cost, simple and requiring minimal maintenance [1, 14, 24, 27, 36, 39, 43, 51-56].

Generally, standard water treatment involves a number of unit processes depending on the quality of water source in question [13], cost and existing guidelines [14, 57, 58], and no one method is devoid of pitfalls [59]. There are some water bodies that are naturally defective due to the geology of the area. For instance, an area with a lot of limestone would produce hard water [19]. In addition, there are also natural and artificial water bodies like ponds that contain a lot of nutrients and unacceptable for consumption. This implies that an appropriate treatment would have to be applied [24, 25]. It has been estimated that 125 liters of water (potable) is required per person per day [4]. However, many households are unable to get 25 liters of clean water per person per day due the high level of contamination. Water purification technologies would have to be reviewed in terms of its simplicity, accessibility (cost) and efficiency. Conventional methods of assuring potable water in developing countries are unsustainable. There is a need to consider the application of sustainable technologies using locally available materials in treating surface water [1]. One sector whose secrets hold a lot of promise for the future is the plant kingdom and particularly the use of Moringa oleifera seeds in water treatment [58-60]. There is a rich bio-diversity of plants that could be explored for sustainable low cost water treatment [67-75]. The search for simple, reliable and effective method of water treatment led to the use of plant materials, including seeds of Moringa oleifera [12, 23, 32, 60]. Despite the increased interest in the application of Moringa oleifera seeds to treat water across Africa pilot systems actually utilizing the seeds are limited. The integration of locally available plant disinfectant and coagulant-Moringa oleifera-sand filter could provide a sustainable alternative water treatment.

3. Poor Household Water Quality in Africa: A Review of Lapses in Current Treatment Applications

Potable water supply at an affordable cost is a major problem in most parts of Africa that needs to be addressed [37]. In urban areas where conventional water is treated using synthetic coagulants and disinfectants, the quality of the water is often
compromised by improper use of chemicals [58, 61]. For example, rota virus in tap water treated with synthetic coagulants and disinfectants has been reported in Ghana [62]. In Cameroon reports in Yaoundé, the capital city indicates that municipal water contains worms [57]. Contamination during storage of treated water and during distribution has been observed [37, 45, 67]. This continues to compromise water quality below the WHO standards [35, 63]. There is a high level of turbidity (greater than 400 NTU) and Coliform counts greater than 10 colonies per mL in surface water often used for household chores [50, 66]. This has been partly caused by runoff during the wet season. Increased population growth and limited land resource, with crops being cultivated in areas closer to the banks of rivers which are reservoirs of water supplies to the cities, is another factor. As a result of this, the soil in this area loosens and is more prone to removal by runoff, thus creating a turbidity problem all year round [1, 50, 67]. This adds to cost of treatment [49]. The lack of suitable technology [67], limited skilled force [31] and insufficient funds has also been identified as part of the problem. Besides, household contamination of water stored in buckets is a major problem in Africa [43]. In Nigeria for instance, a local survey carried out in the states of Bauchi, Plateau and Benue State shows that potable water is unavailable for more than 70% of the rural inhabitants [32] and gets recontaminated at household level. It is believed that a household *Moringa*-sand filter where individual households make their filters using plants cultivated in their vicinity could provide sustainable alternatives to treating any form of turbid water within a relatively shorter residence time and with faster flow rates.


The slow sand filter is inexpensive, easy to install and has been used as an effective means of treating water for more than a hundred years [26, 69]. A combination of biological and mechanical processes is responsible for this. When water is poured on the top layer of the filter bed, the organic material it carries is trapped at the surface of the fine sand forming a biological layer (Schmutzdecke). This biofilm layer matures over a period of three weeks or more depending on the volume of water, the amount of nutrients and microorganisms in the water [61]. A slow sand filter has shown to remove more than 96% of faecal coliforms, 100% of protozoa and helminthes [69] and 50-90% of organic and inorganic toxicants from water [69]. However, the formation of the biofilm layer is crucial and for water with low turbidity effective filtration is compromised [69]. Furthermore, the filtration rate is very slow (0.1 to 0.3 m²/L) and takes more than three weeks for effective filtration [67]. There is considering integration where pretreatment with a one stop coagulant and disinfectant is provided at the same time. On the other hand, natural coagulants and disinfectants have been used for many centuries in Africa to treat water but have their own demerits such as shorter shelf time and high organic matter in the treated water [24, 55, 70].

5. The Applications of *Moringa Oleifera* Seeds in Water Treatment: A Review of Challenges

The seeds from *Moringa oleifera* have proven effective in water treatment [28]. There is growing evidence suggesting that extracts from *Moringa* seed possess both coagulating and antimicrobial properties and are safe for human health [12, 15, 23, 71, 72] as well as heavy metals removal from water [74]. Fig. 1 shows the seeds of *Moringa oleifera* the part that is used as a coagulant in water treatment and the anatomy of the plant showing the pods bearing the seeds. *Moringa oleifera* belongs to the family of Moringaceae, order brassicales and in the division magnoliophyta. Generally, the division comprises of one genus and thirteen species. These include: *Moringa arbores*, *Moringa borziana*, *Moringa concanensis*,
Fig 1 (a) Moringa seeds and (b) plants.

*Moringa drouhardii, Moringa hildebrandtii, Moringa longifolia, Moringa ovalifolia, Moringa pygmaea, Moringa rivan, Moringa rupolinana, Moringa Stemopetala and Moringa oleifera.* Moringa can grow in areas of limited rainfall and poor soil. The plant has five petals sepals, five antepetalous staminodes and five antepetalous stamens with monotheral and bisporangiate anthers. The seeds have a fast germination rate under poor light conditions. It can be sown through the seeds as well as cuttings from the stem of between 1 and 1.5 m in length. A single tree may produce up to 400 to 1000 pods annually depending on the variety and prevailing ecological factors. In villages in Benue state of Nigeria, more than 95% of the houses have a *Moringa* tree. An average of 20 seed bearing pods was observed with an average of 12 seeds per pod [32]. One seed treats a liter of turbid water (with a turbidity of range of 100 to 200 NTU) [46].

Folkard et al. [23] reported that while aerating well water in rural areas of Sudan for reduction of carbon dioxide prior to softening, numerous complaints of red water in hot water systems were received even when aerating was continued and carbon dioxide neutralized with lime in the regular plant treatment process. These complaints ceased and did not reoccur as *Moringa* seeds were used. *Moringa oleifera* is a tropical plant in the family of Moringaceae native to India but found all over the world [24]. It has been described as a miracle tree because the entire plant has amazing uses [60]. The leaves contain vitamin C seven times more than in oranges per gram of dried powder; vitamin A four times more than in carrots, calcium four times more than in milk, potassium three times more than in bananas and protein two times more than in yogurt per gram of dried powder [60]. Furthermore, aqueous extract of the seeds contain antinocarial activity against *Pseudomonas aeruginosa* [32] and against a wide range of gram negative and gram positive bacteria and fungi [76]. The antibiotic protein from *Moringa* seeds have filed for patent [59]. However, care must be exercised when patenting biodiversity. Previous studies demonstrate that *Moringa* seed possess a bioactive protein (*Moringa* Coagulant Protein-MOCP) responsible for turbidity removal from water [24, 28, 41]. However, a lot of constraints exist on a commercial application of *Moringa* seed extracts in large scale water treatment systems [43]. *Moringa* seed treated water cannot be stored for more than 24 hours. This is because *Moringa* seed generates a lot of organic carbon in water thereby stimulating regrowth of bacteria after 24 hours. Treating large volumes of water requires a longer hydraulic retention time and using *Moringa* as a coagulant is limiting. This has provoked several studies with interest in isolating and purifying the bioactive coagulant protein [14, 15, 17, 24, 77, 78]. These studies have been carried out to clarify the performance of MOC extracted with different methods.
especially with salts [79]. Most of these studies, however, suggest that the active coagulant ingredient is a protein [14, 17], but other reports exist to suggest that the coagulant ingredient is a water soluble lectin and antioxidant [80, 81]. Peptides have been reported by Prasad et al. [70] in their studies which demonstrated that it is neither a protein nor a polysaccharide but an organic polyelectrolyte [13]. Although *Moringa* seed is a good coagulant, like the slow sand filter, is only very effective with highly turbid water [26]. Furthermore, more studies on the disinfection property of *Moringa* seed extracts are necessary. There have been more studies on *Moringa* coagulant activity for more than three decades [24, 82, 83], but these studies are increasingly tailored toward the isolation and purification of the active components with so far few field applications to treat the soaring water crisis in Africa have been developed [24, 25, 82, 84]. It is strongly advocated that integrating *Moringa* seed powder into a sand filter system could treat any type of turbid water with a faster flow rate and shorter residence. Research in this aspect is necessary.

6. Conclusion: Future Research Focus on *Moringa*-Sand System

Every society evolves with its own problems, but there could be latent solutions behind the problem. In this research, it is concluded that the solutions to the water crisis lay in the appropriate exploitation of the inherent resources through an appropriate application of science and technology [27, 84, 85]. The documentation of indigenous plants with potentials for water treatment such as in local Bolivia where poor water quality was a problem has been reported. Cactus was reported as an indigenous coagulant in Bolivia where the indigenous people grew up solving their water problem [56]. Such studies are very necessary on every continent and should be continuously documented and validated. Practical translation of such knowledge to field benefits is more important. Plants with potential to remove turbidity and nutrients from water and wastewater [64, 85] and disinfect microorganisms could be selected for use with the slow sand filter system. The *Moringa* integrated sand filter system if carefully studied and implemented could treat all types of turbid and wastewater. It is also expected that a 100% disinfection rate, faster flow rates and shorter residence time with little clogging and backwashing of filter may be the potentials of this hybrid system. It is thus concluded that future research should also focus on integrating *Moringa oleifera* with sand filter to treat water within a relatively shorter residence time.

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Chapter 4

A Moringa Oleifera Disinfectant-Sand Filter Integration: A Review of an Alternative Sustainable Technology for Household Water Treatment

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Chapter 4

A Moringa Oleifera Disinfectant-Sand Filter Integration: A Review of an Alternative Sustainable Technology for Household Water Treatment


CHAPTER 5

APPLICATION OF PHOTODISINFECTANTS IN WATER TREATMENT IN RURAL CAMEROON

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Article 2 is presented in this chapter. The application of *Moringa oleifera* seeds, *Aloe vera* and *Jatropha curcas* seeds from rural Cameroon are screened for efficacy in disinfecting turbid water. In this article, the invitro antimicrobial activities of the extracts from these plants on microbes isolated from surface water demonstrate appreciable efficacy. The turbidity removal and toxic effect on microbes using surface water from Cameroon is applied. The results obtained are comparable to chlorine and alum. The terms phytodisinfectant and phytocoagulants are novel terms used for the first time in this publication and can be found in Google linked to this research. The general recommendations in this paper are in tandem with a longtime traditional practice of using plants to purify water.

This article, cited more than 100 times, is published in the African Journal of Microbiology Research in 2011, vol 5, issue 6, pp 628-635, a top journal in Africa with a reasonable impact factor on the African continent, widely abstracted in PubMed, CABI bioscience and widely cited worldwide.

![Figure 20: Anti bacterial activity of M. oleifera extracts on A. hydrophila and E. coli](http://hdl.handle.net/2440/69910)
Full Length Research Paper

Application of phytodisinfectants in water purification in rural Cameroon

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Findings from a preliminary lab-scale study show strong potentials of phytodisinfectants as a low-cost, appropriate and ecological alternative technology in purifying water in rural Cameroon. A survey of plants used in water purification in Bamenda, Cameroon, indicated that there are many plants used in water treatment. A rapid screening on the coagulative and disinfection potential of four most frequently used plants was carried out on: Morinda oleifera, Jatropha curcas, calyx of Hibiscus sabdariffa, sclerotium of Pueraria tuberorum against their crude methanol extracts, aluminum sulphate and sodium hypochlorite controls on turbid surface water samples. A beaker experiment with varying weights (0.5 to 5 g) of dried pulverized plant materials and alum (control) were placed in 200 ml each of the three-turbid water samples and left for thirty minutes retention time. A 95% reduction in bacterial loads of the water samples by M. oleifera in fifteen minutes residence time was observed. J. curcas seeds, as well as H. sabdariffa calyx also reduced the bacterial loads between 75 to 90%. All the plant extracts except P. tuberorum inhibited an Escherichia coli isolate from the turbid water with highest zone of inhibition (15 mm) recorded for M. oleifera seed extract. The inhibition zones produced by three of the plant extracts were comparable to aluminum sulphate (6 mm) and sodium hypochlorite (17 mm). Crude methanol extracts from M. oleifera seeds, J. curcas seeds and H. sabdariffa calyx used directly on turbid water drastically reduced the total aerobic mesophilic bacterial counts far more than the unextracted plant powders. The turbidity of both phytodisinfectant and alum treated water samples drastically reduced while no turbidity reduction was observed with sodium hypochlorite treated water samples. The pH of alum treated water was observed to decrease from neutral to 5.0 while pH of phytocoagulant treated water was 7.0. This report suggests that M. oleifera seeds, J. curcas seeds and calyx of Hibiscus sabdariffa possess both phytodisinfectant and phytocoagulant property in water purification. Sclerotium of P. tuberorum poses only phytocoagulant (mycococagulant) activity. Plant materials can be used as phytocoagulants and phytodisinfectants in treating turbid water and can be applied in wastewater treatment. Further studies on the application of Phytodisinfectants in domestic water purification, especially the phytodisinfection potentials of M. oleifera are exigent.

Key words: Phytodisinfectants, phytobiotechnology, phytocoagulants, bacteria turbid water, plants, Morinda oleifera, Cameroon.

INTRODUCTION

Safe drinking water and adequate sanitation are essential for human health and dignity. However, 1.2 billion people do not have (Pritchard et al., 2009; UNICEF, 2009). Approximately, 2.5 billion people in the world lack adequate sanitation facilities (UNICEF, 1993; UNEP, 2002; Zhang et al., 2006; UNESCO, 2007; UNICEF, 2009). Waterborne and water related diseases such as diarrhea, typhoid, cholera and dysentery are fast becoming endemic in certain parts of Africa (Chasebrough, 1984; Yongabi, 2004; Pritchard et al., 2009). Yet, the present well documented technologies used in water treatment such as reverse osmosis, ion exchange, uv sterilization, aluminum sulphate and chlorine are becoming unsustainable, unecological,
efforts on taking inventory of other potential plant coagulants and disinfectants. The most important step in water treatment is disinfection. Attention has been focused on screening plants for coagulant activity (Ellert, 1978; Jahn, 1981; Muyibi et al., 2002a; Kebreab et al., 2005; Amir et al., 2010), but not all coagulants are disinfectant. The flora of Africa is rich with a lot of medicinal plants and Macro fungi which people in the rural areas are quite familiar (Figure 2). Solowora (1982) and Yongabi (2004) reported that Africa has as much as 300, 00 medicinal plants while Chang (1993) reported that the world mushroom biodiversity is as much as 1.5 million species. There is, therefore, an urgent need to explore and utilize these rich biodiversity through researches that could translate to direct benefit to humankind (Yongabi, 2009). Plant disinfectants could provide useful insight for the production of natural disinfectants and coagulants which are environment friendly and with much reduced risk of handling.

The ultimate purpose of this research was to carry out an inventory of plants used as disinfectants in rural Cameroon, conduct an in vitro evaluation of crude plant powders and solvent extracts on directly disinfecting turbid surface water from Bambui, Cameroon.

MATERIALS AND METHODS

Water sample collection

Water samples collected included turbid surface water sources in Bambui, Cameroon. Dirty water flowing in Gullies and water from a stream around the Bambui settlement, kitchen sink samples, as well as septic tank outflows was collected. The appearance/cloudiness of these water samples were noted by visual observation (Burra and Van, 1974).
Chapter 5


pH analysis
The pH was recorded using a test strip (combi 9 test strips) used at the phytotechnology research laboratories for routine urinalysis.

Microbial analysis of untreated and treated water samples
*Escherichia coli*, coliforms and total aerobic mesophilic bacterial counts were enumerated on Eosin Methylene blue, MacConkey and Nutrient agar respectively (Harrigan and Mocanu, 1978). A milliliter of the turbid water samples were aseptically diluted serially up to three fold. This was done according to methods of APHA (1983), Chessbrogh (1984) and Yongabi (2004). All these were done before and after coagulation and disinfection.

Plant collection and processing
Seeds of *M. oleifera*, *Jatropha curcas*, calyx of *Hibiscus sabdariffa* and *Scirrorum of P. tuberiferum* were previously obtained from Fulani settlements around Sabga in the North West Region of Cameroon, dried, then pulverized separately using a clean pestle and mortar and stored in brown paper bags ready for the test. The identities of the plants were previously confirmed by Botanist at The University of Yaounde, Cameroon.

Beaker tests to determine coagulation and coagulant using crude plant powders
Graded weights (0.59 to 59) of the pulverized plant materials and Alum were all added to 200 ml of the wastewater samples in beakers. Increased weight s (g) from 0.5 to 5.0 g of each of the plant material was mixed in a small quantity of the turbid water to form a paste and then mixed finally with the water samples in the beakers. The same procedure was done for alum, sodium hypochlorite and a turbid water sample in a beaker (200 ml) was allowed to stand in a beaker for 24 h as control. The coagulative effect and change in bacterial counts were recorded (Kebreab, 2005; Pichard et al., 1996).

Bulk methanol extraction procedure for plant powders
A cold methanol extraction was then carried out on 100 g each of the powders by stirring each of the powders in 250 ml of methanol for 24 h. Gravity filtration was carried out using whatman filter paper No. 13 and solvent evaporated at room temperature in a fume cupboard.

Preliminary antibacterial determination of crude methanol extracts on an E. coli isolate from surface water in Bambui, Cameroon
This was carried to confirm if the bacterial reduction previously observed is due to inherent disinfectant ability of the plants. One hundred (100) mg of each of the extract was suspended in 1 ml of distilled water. The extracts were now tested for their *in vitro* antibacterial activity using the Agar diffusion Method using *E. coli*, previously isolated from the turbid water rather than using synthetic turbid water as previously carried out by most researchers. The choice of *E. coli* is because *E. coli* are an important indicator of faecal contamination of water. A 100 mg of Alum and sodium hypochlorite was also tested against *E. coli* as control. The whole set up was incubated at 37°C for 24 h and the diameter zone of inhibition was recorded in millimeters.

Test tube *in vitro* coagulation and disinfection test using methanol extracts of the plant materials
This was a novel technique meant to further proof phyto-disinfection activity of the extracts. This technique has not been reported elsewhere preliminary *in vitro* coagulation and disinfection studies was also carried out using the methanol extract from each of the plants. Zero point five (0.5) ml of each of the extract was dropped in 5 ml of Turbid water in a test tube, clearance rate after 10 to 15 minutes residence time was reported visually and a ml of each of the treated water was cultured for total bacterial counts (Yongabi, 2004).

RESULTS AND DISCUSSION
The list of plants used as phyto-disinfectants, availability, growth conditions and coagulants in rural Cameroon is shown in Table 1. The plants are very widespread across Africa and in a poor soil as well as different climatic conditions. The result of the physical nature and microbial content of turbid water samples from Bambui, Cameroon before Treatment with Alum and plant materials is presented in Table 2. The total aerobic mesophilic bacterial counts, *E. coli* counts as well as coliform counts were generally high, especially in stagnant, dirty stream water and samples collected from gutters or drainage where microbial counts were too numerous to count (NTNC). Wastewater in Bambui neighbourhood in Bamenda is not properly disposed. There is a possibility the wastewater discharged from homes contaminate surface and ground water use for drinking and domestic chores. A similar observation in Yelwa community in Bauchi, Nigeria was previously reported (Yongabi, 2004). The results in Table 2 served as untreated control all through the experiment. The pH of the turbid water samples was 7.0 using a urinalysis test strip - Combii 9 test strip while the turbidity which was assessed subjectively using visual observation was noted to be highly turbid with an unpleasant odour for most of the samples except the surface water. This observation has been previously reported for water in a neighbouring in Bambui; Nigeria (Yongabi, 2004).

Table 3 shows the results of the coagulative and disinfective effect of *M. oleifera* seeds on turbid water in comparison with aluminium sulphate.

The findings indicated that *M. oleifera* seeds coagulated well above 90% of the particles in the samples leading to a clear supernatant. The coagulation effect was far better in heavily polluted water than in less polluted water and this observation has been previously noted (Yongabi, 2004). Unlike with alum, floc settlement in *Moringa* treated samples was slower when the seeds were directly dispersed into the water but when the seeds were packed in to a muslin sackcloth and dipped into the samples, floc settlement was faster and was as good as with Alum and has been previously observed by Prasad (2009). The coagulative effect of *Moringa* seeds was even better than with Alum and this can be explained with
Table 1. List of plants used as phytodiesinfectants and coagulants in rural Cameroon.

<table>
<thead>
<tr>
<th>Plant material</th>
<th>Moringa oleifera</th>
<th>Aloe barbadensis</th>
<th>Jatropha curcas</th>
<th>Pleurotus tuberregium</th>
<th>Citrus aurantifolia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family/ Local name botanical name</td>
<td>Horse Radish Zobala (Hausa) Moringa Oleifera Lam Moringaceae</td>
<td>Aloe Vera Aloe Lilliacae</td>
<td>Euphorbiaeae Physic Nut Benin Zugo (hausa)</td>
<td>Pleurotus tuberregium fr. Singer Mushrooms</td>
<td>Lime</td>
</tr>
<tr>
<td>Part used</td>
<td>Seeds</td>
<td>All types of soil but mostly alluvial sandy soil, hot low lying semi arid areas</td>
<td>All types of soil and survives in different climates</td>
<td>Tropical and sub tropical</td>
<td>Temperate and tropical conditions</td>
</tr>
<tr>
<td>Climatic conditions</td>
<td>Very available across Africa</td>
<td>Very available across Africa</td>
<td>Very available across Africa</td>
<td>Limited to tropical areas</td>
<td>Yield high in the tropics</td>
</tr>
<tr>
<td>Cost of plant material</td>
<td>1 kg of seeds in 5000 naira in Cameroon and 40 naira in Nigeria</td>
<td>Hard to determine as most people grow it</td>
<td>Not determined</td>
<td>1 KG of sclerodium cost 5000 naira in Cameroon</td>
<td>1 KG of limos cost 2000 naira in Cameroon.</td>
</tr>
<tr>
<td>Availability and yield</td>
<td>Found all over Cameroon and across Africa, Average seeds per pod and 1 seed purifies a litre of turbid water</td>
<td>No published work on its use in water purification</td>
<td>Literature exist in its use in water (Yongabi, 2004; Pritchard et al., 2009)</td>
<td>Cultivation of sclerofia takes long</td>
<td>Impart Specific lime odour and renders water acidic</td>
</tr>
<tr>
<td>Limitation</td>
<td>Treated water must be used in less than 2 days</td>
<td>Impart a slightly bitter taste in water</td>
<td>Seeds, toxic</td>
<td>Little report on used as a coagulant</td>
<td></td>
</tr>
<tr>
<td>Remarks from literature survey</td>
<td>Extensive literature in the plant with many tribes aware of its uses. One of the best plant coagulants known. Studies on disinfection limited.</td>
<td>No published work on its use in water purification</td>
<td>Literature exist in its use in water (Yongabi, 2004; Pritchard et al., 2009)</td>
<td>Cultivation of sclerofia takes long</td>
<td>Impart Specific lime odour and renders water acidic</td>
</tr>
</tbody>
</table>

the fact that M. oleifera seeds exhibited strong antimicrobial activity. The raw untreated stagnant water from the gutters or drainage had an initial total bacterial counts Too Numerous to count, which reduced to only 4000 colony forming units per ml when treated with Moringa seeds as opposed to 11000 colony forming units when treated with alum. Alum exhibited a minimal effect on bacterial load of the samples. This may be partly due to the fact that some bacteria attach to the surface of particles that eventually settles with them and as well as the acidic nature of alum might have had some influence on the bacterial counts. To further prove this point, a 100 mg/l of alum was tested on E. coli isolated from the surface turbid water samples and only a traceable inhibition (less than 1 mm) was noticed as opposed to marked inhibition demonstrated by M. oleifera (15 mm) and chlorine (17 mm). Meanwhile a 100 mg/ml of (bulk) methanol extract of M. oleifera seeds when tested
Table 2. Physical nature and microbial content of turbid water samples from Bambui, Cameroon before treatment with alum and plant materials.

<table>
<thead>
<tr>
<th>Type of waste Water sample Counts (CFU/ml)</th>
<th>Physical appearance</th>
<th>Total aerobic Mesophilic bacteria (CFU/ml)</th>
<th>Coli form counts</th>
<th>Escherichia coli counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dirty water Stagnant in Gutters Lot of suspended Matter</td>
<td>Very dirty and highly turbid with a</td>
<td>TNTC</td>
<td>TNTC</td>
<td>TNTC</td>
</tr>
<tr>
<td>Dirty, heavily polluted stream With garbage dumps</td>
<td>Very turbid appearing brownish</td>
<td>800000</td>
<td>52,000</td>
<td>31000</td>
</tr>
<tr>
<td>Kitchen sink samples from Bambui</td>
<td>Dirty, cloudy and appearing very turbid</td>
<td>650000</td>
<td>47,200</td>
<td>6000</td>
</tr>
</tbody>
</table>

Key: CFU---- Colony forming unit per ml, TNTC ------- Too numerous to count.

Table 3. Coagulative and disinfective effect of Moringa oleifera seeds and alum on turbid water samples.

<table>
<thead>
<tr>
<th>Type of water sample</th>
<th>Physical appearance (Arbitrary classification)</th>
<th>Treatment with alum (Aluminum sulphate) (5 g)</th>
<th>Treatment with Moringa Oleifera seeds (5 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dirty water</td>
<td>Very dirty</td>
<td>Flocs settled</td>
<td>Flocs formed and settled when directly dispersed in water</td>
</tr>
<tr>
<td>Stagnant in gutters</td>
<td>Very turbid with a lot of suspended particles</td>
<td>Clear supernatant</td>
<td>Well settled when held in muslin cloth</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ec = 4500 CFU/ml</td>
<td>Supernatant clear</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ct = 3500 CFU/ml</td>
<td>Ec = 1500 CFU/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TBC = 11000 CFU/ml</td>
<td>Cf = 5200 CFU/ml</td>
</tr>
<tr>
<td>Dirty heavily polluted stream with garbage in Bambui</td>
<td>Very turbid brownish</td>
<td>Flocs formed + settled</td>
<td>Flocs well settled when held in muslin sack cloth, clear supernatant,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ec = 2375 CFU/ml</td>
<td>Ec = 2190 CFU/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ct = 47,900 CFU/ml</td>
<td>Cf = 6100 CFU/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TBC = 720,000 CFU/ml</td>
<td>TBC = 73,000 CFU/ml</td>
</tr>
<tr>
<td>Kitchen sink samples from Bambui</td>
<td>Dirty, cloudy + turbid</td>
<td>Flocs settled</td>
<td>Flocs settled</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ec = 3152 CFU/ml</td>
<td>Ec = 800 CFU/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ct = 5000 CFU/ml</td>
<td>Cf = 3215 CFU/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TBC = 121,000 CFU/ml</td>
<td>TBC = 61000 CFU/ml</td>
</tr>
</tbody>
</table>

showed an appreciable inhibition on the same E. coli. These results are shown in Table 6. The coagulative property of Moringa seeds could be attributed to a polymeric coagulant earlier reported by Ellert et al. (1978) and Kebreab (2005). The works of Ellert (????) also supports the antibacterial activity of
Table 4. Phytoocoagulative and Phytodisinfective effects of Jacarpha curcas seeds, Pleurotus tuberregium sclerolium, Hibiscus sabdarifa calyx and Alum on turbid water samples.

<table>
<thead>
<tr>
<th>Type of water sample</th>
<th>Physical appearance</th>
<th>Alum treatment (control)</th>
<th>Treatment with Jacarpha curcas seeds</th>
<th>Treatment with Pleurotus tuberregium Sclerolium</th>
<th>Treatment with Hibiscus sabdarifa calyx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dirty water stagnant in Gutters</td>
<td>Dirty, Very turbid and suspended particle</td>
<td>Flocs settled clear supernatant Ec = 3150 cfuml CF = 4982 cfuml TBC = 9300 cfuml</td>
<td>Flocs Formed and settle, clear supernatant Ec = 2254 cfuml CF = 4486 cfuml TBC = 6115 cfuml</td>
<td>Flocs formed slowly settled supernatant Ec = 3521 cfuml CF = 4250 cfuml TBC = 6100 cfuml</td>
<td>Flocs formed settled slowly, red pigment extracts, Clear Ec = 2819 cfuml CF = 13516 cfuml TBC = 52,150 cfuml</td>
</tr>
<tr>
<td>Dirty heavily polluted brownish stream at Yelwa, Bauchi colour</td>
<td>Very Turbid</td>
<td>Flocs settled, Very clear supernatant Ec = 18250 cfuml CF = 39910 cfuml TBC = 61500 cfuml</td>
<td>Flocs settled clear supernatant Ec = 5012 cfuml CF = 8300 cfuml TBC = 232,000 cfuml</td>
<td>Flocs slowly settled, clear supernatant Ec = 16000 CF = 3030 TBC = 209000</td>
<td>Flocs formed slowly settled red pigment observed Ec = 3522 cfuml CF = 4252 cfuml TBC = 52180 cfuml</td>
</tr>
<tr>
<td>Kitchen sink sample from yelwa</td>
<td>Dirty cloudy turbid</td>
<td>flocs settled supernatant Ec = 3567 cfuml CF = 3675 cfuml TBC = 617000 cfuml</td>
<td>Flocs formed settled, clear supernatant Ec = 1115 cfuml CF = 4500 cfuml TBC0108000 cfuml</td>
<td>flocs formed + settled, clear, supernatant Ec = 3526 cfuml CF = 43000 cfuml TBC = 58100 cfuml</td>
<td>Flocs formed E settled slowly supernatant but red Ec = 440 cfuml CF = 2561 cfuml TBC = 39725 cfuml</td>
</tr>
</tbody>
</table>

EC—Escherichia coli, CF—Coliform, TBC—Total bacterial counts, CFU/ml—Colony forming units per ml.

Moringa oleifera, while Umar Dahot. (1996) and Kebreab (2005) reported the antibacterial action of small protein/ppeptide against E. coli, Klebsiella aerogenes, Klebsiella pneumoniae, S. aureus and Bacillus subtilis. The observations in this study corroborates these earlier observations in that M. oleifera seeds reduced the total aerobic mesophilic counts drastically and the bacterial isolates listed in their studies are actual aerobic mesophilic bacteria (Amir et al., 2010). A better coagulation and disinfection activity was observed with the methanol extracts of the plants. This corroborates previous views that pure extracts of plant seed powders such as M. oleifera possess better coagulant and disinfection activity than the plant materials (Kebreab, 2005; Amir et al., 2010).

The results of the coagulative and disinfective effects of J. curcas seeds (Physic nut), Pleurotus tuberregium sclerolium, H. sabdarifa calyx and alum on turbid water samples are shown in Table 4.

The results generally show that the plant materials possess some disinfective and coagulative effect with M. oleifera seeds; J. curcas seeds and calyx of H. sabdarifa possess both coagulative and disinfective ability. Generally in the experiment, the weight of the plant materials and alum were varied from 0.5 to 5 g per 200 ml turbid water samples. The best coagulative effect was noted between 4 to 5 g plant powders. The result of the experiment was reported using 5 g and previous observed by Yongabi (2004).

When compared to the untreated turbid water samples, all the plant materials except M. oleifera (>95%) are between 60 to 90% effective in purifying the water samples used in the study. The coagulative effect of the mushroom sclerolium of P. tuberregium was the least of all the plant materials. The first report on the use of this mushroom in water treatment was reported by Yongabi (2004) who observed a similar trend as in this study. The mild effect on the bacterial load of the turbid water may be explained in the fact that extracts of P. tuberregium do not possess any antibacterial activity (Tables 5 and 6).

The mild reduction of bacterial load is possibly due to the fact that settled particles in water do have organisms attached to their surfaces. This effect is possibly the case with alum.
Table 5. Preliminary antibacterial activity of methanol extracts of plants and alum on Escherichia coli isolated from waste water samples (mm).

<table>
<thead>
<tr>
<th>Extracts alun (100 mg/ml)</th>
<th>Sensitivity on E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol extract of Moringa Oleifera seeds</td>
<td>15</td>
</tr>
<tr>
<td>Methanol extract of seed of Janophya curcas</td>
<td>8</td>
</tr>
<tr>
<td>Methanol extract of calyx of Hibiscus subdarifa</td>
<td>14</td>
</tr>
<tr>
<td>Methanol extracts of sclerotium of Pleurotus tuberregum</td>
<td>0</td>
</tr>
<tr>
<td>Alum (aluminum sulphate)</td>
<td>6</td>
</tr>
<tr>
<td>Sodium hypochlorite</td>
<td>17</td>
</tr>
</tbody>
</table>

Table 6. Disinfection effect of methanol extracts of three plant materials on total bacterial counts of turbid water in CFU/ml.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Initial total counts</th>
<th>Total bacterial after</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol extracts of Moringa</td>
<td>TNTC</td>
<td>100</td>
</tr>
<tr>
<td>Methanol extract of aleo barbadensis gel 85CFU/ML</td>
<td>TNTC</td>
<td>85</td>
</tr>
<tr>
<td>Methanol extracts of sclerotium 750CFU/ML of Pleurotus tuberregum</td>
<td>TNTC</td>
<td>7500</td>
</tr>
<tr>
<td>Sodium hypochlorite 50CFU Control</td>
<td>TNTC</td>
<td>50</td>
</tr>
</tbody>
</table>

TNTC—Too numerous to count.

The mushroom sclerotium powders coagulated the particles better when placed in muslin sackcloth. This was generally observed for all the plant materials and this is because pulverized plant materials had very small particles that remained in colloidal form. The use of sclerotium of the mushroom P. tuberregum to purify water can lie in the ground for years and during which it fruits repeatedly at the onset of the rains. In Igbo land in Nigeria as well as in the Bayangi and Bakwiri clans in Cameroon the pulverized sclerotium is used in the preparation of a soup delicacy that is well valued by notables in these societies. There are many plants with plants with phytocoagulant and disinfectant ability that needs to be exploited (Table 1).

The seeds of J. curcas exhibited a stronger coagulative as well as disinfactive effect than Alum. (Tables 4 and 5) but when compared to M. oleifera is about 60 to 80% while Moringa was above 95%. J. curcas is a very common plant with a number of medicinal uses. The root extracts have been used in the treatment of sexually transmitted diseases while the leaf latex has been used as a hemostat (Yongabi, 2004). The use of the powders of the calyx of H. sabdarifa has shown both coagulative as well as disinfactive effect. It reduced total aerobic bacterial counts, E. coli and coliform counts greater than Alum but generates a red pigment extract in the water leaving the water colored. The methanol extract of H. sabdarifa calyx demonstrated antibacterial activity (Table 5). The calyx extract of H. sabdarifa has been widely used as a local beverage in Northern Nigeria.

Generally, the turbidity of the water samples reduced drastically after treatment with all the plant materials. Alum decreased pH of the turbid water samples from 7.0 to 5 while with the pH of plant materials treated, and in Malawi where two grams of the crushed seeds has been used to treat 20 L of water but the use of other plants especially those specified in this study have not been known nor reported elsewhere except in an earlier report of Yongabi (2004).

This study has conclusively indicated that turbid water can be treated considerably with the application of phytodisinfectants and phytocoagulants. The inhibitory effect of M. oleifera seeds extracts, J. curcas seed extracts and H. sabdarifa on E. coli isolates as well as the drastic reduction of bacterial load from the turbid water demonstrates that these plants are phytodisinfectants. All the plants studied showed phytocoagulant activity but not phytodisinfectant ability. This research has also shown that there are many plants that need to be screened properly for water treatment (Table 1). The need to exploit the potential of plants may offer cheap, and environment friendly methods of tackling water contamination and may possibly overcome the hazards of using synthetic compounds. From this study, further studies on the actual application of M. oleifera seeds in water purification at household or large scale level in
Africa is required.

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CHAPTER 6

INDIGENOUS PLANT BASED COAGULANTS AND SAND FILTER MEDIA FOR SURFACE WATER TREATMENT IN BAMENDA, CAMEROON

Yongabi¹, K.A., Lewis¹, D.M and Harris² P.L

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2. School of Agriculture, Food and Wine, the University of Adelaide

In this chapter Article 3 is presented, which examines a plethora of indigenous plants other than Moringa oleifera seeds in water treatment. This indigenous knowledge is validated and such information given a scientific background. The short falls of the use of these plant materials in water treatment are overcome with the integration onto a sand filter and efficacy tested using a range of water. The article was published in the African Journal of Biotechnology Vol. 10(43), pp. 8625-8629, 10 August, 2011 Available online at http://www.academicjournals.org/AJB ISSN 1684-5315 © 2011 Academic Journals - Full Length Research Paper.

This Journal is the most prominent in biotechnology in Africa, with a reasonable impact factor and widely cited and abstracted.

Figure 21: Indigenous African plants used to purify water (Carica papaya and Jatropha)

Full Length Research Paper

Indigenous plant based coagulants/disinfectants and sand filter media for surface water treatment in Bamenda, Cameroon

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An Evaluation of plant-based coagulants and disinfectant-sand filter medium for surface water treatment in Bamenda, Cameroon using bacterial analyses and turbidity were carried out. 100L of very turbid surface water (Turbidity approx. 500NTU) was pretreated with 100 seeds of Moringa oleifera, and further filtered through a sand filter drum (120 L carrying capacity) made of fine, coarse sand, charcoal and gravel. The mean total heterotrophic bacterial counts, Escherichia coli, coliform, pseudomonas and yeast counts, as well as turbidity of untreated surface water significantly reduced by 85 to 95%. The results suggested that the mean values of the same parameters for sand filtered pond water alone was significantly lower than the corresponding mean values obtained for plant coagulant treated surface water. The findings from this study demonstrates strongly that a biocoagulant sand filter media (plant based coagulant-sand filter drum) could be applied to treat contaminated surface water, rendering it free from solids and pathogens.

Key words: Plant, coagulants, indigenous, surface, water, treatment, microbes, Cameroon.

INTRODUCTION

Water remains a strategic resource for the integration of economic, social and environmental concerns and it is key to sustainable development. It sustains human productivity and livelihoods and plays a crucial role in integrating world’s ecosystems. Water is under increasing and competing demands from agricultural, industrial and domestic uses with increasing pollution threatening this scarce resource. Approximately 1.6 million people are forced to use contaminated water globally. Uncontaminated water is rarely obtainable in rural Africa, Asia and especially in sub Sahara Africa (SSA). The prevalence of waterborne infectious diseases in SSA is rising (Yongabi, 2004; Yongabi et al., 2009). Waterborne diseases contribute to the death of about 4 million children in the developing countries per annum. The world health organization has estimated that up to 80% of all diseases and sicknesses in the world is caused by inadequate sanitation, polluted water or unavailability of water (Pritchard et al., 2009). It is beneficial to treat water both for domestic use and safe disposal to the environment.

Water scarcity remains intractable in sub-Saharan Africa, especially in rural, semi-urban and even urban areas in these countries. There are also natural and artificial water bodies like ponds that contain a lot of nutrients that are unacceptable for consumption. Approximately 125 L of clean water is required per person per day, yet, many households cannot actually boast of 25 L of clean water per person per day (Pritchard et al., 2009). To this effect, water purification technologies would have to be considered with respect to efficiency and cost.

The need for simple, reliable and effective method of water treatment led to the application of plant materials, including seeds coagulants of Moringa oleifera (Elet, 1978; Yongabi, 2004; Ghebremichael et al., 2009; Pritchard et al., 2009). Standard methods for the treatment of water include coagulation, flocculation, sedimentation, disinfection, membrane filtration, reverse osmosis...
and UV. These methods are often inappropriate in SSA due to prohibitive cost and scarcity of chemical coagulants and disinfectants. Dosage and technique pose some local challenges, and for these reasons, efforts to establish appropriate chlorination techniques for wells in rural communities is imperative. These technologies presently require high energy inputs (Fuglie, 1999).

Most communities in sub Saharan Africa lack electricity and this makes it difficult to have a water treatment technology that depends on electricity. Water purification using seeds of *M. oleifera* plant has been reported extensively (Fuglie, 1999; Folkard et al., 2000). Bioactive coagulant proteins have been characterized (Ghebrebrhan, 2009) and pretreated plant coagulants moringa and sand filtered water may overcome the limitations of both techniques. Little attempts to document and screen other plant-based coagulants, other than moringa have been made. This study was therefore designed to investigate the potential indigenous plant coagulants other than *M. oleifera* seeds and sand filter media for surface water treatment in Cameroon. The ultimate purpose of this study was to come up with a compendium of plant coagulants that could be used as a technology that is low tech, effective and ecological. This report presents the potential results of integrating phycocoeagulants using *Garcinia kola* and *Carica papaya* seed powder and sand filtered treated surface water.

**MATERIALS AND METHODS**

**Plant collection and processing**

Mature seeds of *G. kola* and *C. papaya* were obtained in Bamenda. Carica papaya 100 seeds were desiccated and pulverized in clean mortar using a pestle. The powder (from 100 seeds) was sprinkled onto 100 L of the dirty pond water in a 150 L capacity drum and stirred using a clean wood stirrer and the set-up was allowed to stand for 30 min (Folkard et al., 1995). It was then filtered off using a muslin sack cloth and the filtered water was then passed through a sand filter drum. The materials used in the construction of the sand filter were locally gotten at a river bed and included: few strong (150 L carrying capacity) drum (plastic), 1½ yards of hose, four clips, three nipples, strainer or sieve, sharp river sand (coarse and fine), charcoal and gravel. All these materials (sand, gravel and charcoal) were carefully washed and rinsed repeatedly in clean water (Folkard et al., 1990).

The laying of the materials in the drum was done in the order: layering of perforated hose connected to the collector tank, than a layer of gravel, followed by a layer of charcoal, then coarse sand (2 mm in size) and two layers of fine sand (0.15 to 0.30mm size). A test trial was carried out by flushing the set up repeatedly with clean water. The moringa pretreated water was then passed through the system. The filtered water was collected into the drum and three samples each were taken for analysis. Three water samples each (stream from Milu 6 mankon, Bambui and Storm water) were collected and subjected to all these treatments. The surface water was located in mile 6 Mankon, Bambui and Storm water. The surface water was shallow, very turbid and during the dry season, people sometimes fetch the water for household chores, stray animals, and cows drink from the source. Storm water was collected using sterile bottle from the drains after heavy rains.

**Turbidity**

The turbidity for each of the water samples was read before and after treatment using a turbidity meter (HACH DR, 2000).

**Microbiological assessment of the surface water**

The microbial analysis was carried out according to the protocols specified by Burns (1974), APHA (1983) and APHA and WEF (1995). The MPN technique was avoided due to the fact that it is time consuming, while the membrane filter technique was considered because of certain limitations such as lack of facility for coliform counts and total aerobic bacterial counts etc. While the presumptive coliform test is also time consuming as limited in other microbial parameters. A range of tests such as total heterotrophic bacterial counts, *Pseudomonas* aeruginosa counts, as well as yeast counts were considered in addition to the traditional coliform and faecal coliform tests to explore the possibility of taking care of viable but non culturable bacteria. The traditional indicator tests do not always correlate well with certain groups of pathogens such as *Helicobacter pylori* and *Aeromonas* spp. and it was worthwhile considering this, especially in assessing the potential of newly constructed systems for water purification as well as screening for new phycocoeagulants.

**Culture tests**

Three samples from each of the surface water samples were collected using sterile MacNabney bottles. 1 ml of the samples each was serially diluted with distilled water (9 ml) three fold up to 10⁻³ and 1 ml of each diluent of 10⁻¹ and 10⁻² were plated aerobically onto nutrient agar for total heterotrophic bacterial and pseudomonas counts, MacConkey agar for total coliform, eosin methylene blue agar for *Escherichia coli* counts and potato dextrose agar for yeast/fungal counts (Christbough, 1984). Incubation was carried out at 37°C for 24 h, and the plates were read following standard microbiological procedures. The bacterial counts were enumerated using Gallippen colony counter and recorded accordingly, while the yeast and pseudomonas colonies were picked, stained using methylene blue and gram stain, respectively. The average counts from 10⁻³ and 10⁻⁴ dilutions were recorded (Ellis, 1988; Yonjabi, 2006; Yonjabi et al., 2010).

**RESULTS AND DISCUSSION**

The results are presented in Tables 1 to 6. The findings suggest the combination of *G. kola* and *C. papaya* seeds with the sand filter outfit purified surface water. The raw data obtained from the surface water in Bamenda showed a very high total heterotrophic bacterial population and high faecal indicator bacteria, suggesting the presence of pathogens. This observation was observed in surface water in Malawi (Pritchard et al., 2009).

Surface water is not usually consumed in Bamenda but during the dry season when there is acute water shortage, people fetch such water for other non potable domestic uses and occasionally boil it for drinking. In rural Cameroon there is water scarcity. The high E.
### Table 1. Mean total bacterial and fungal counts of surface water in Cameroon before treatment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Stream 1 (mile 6 Mankon)</th>
<th>Stream 2 (Bambui)</th>
<th>Water source 3 (stormwater)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coliform counts (cfu/ml)</td>
<td>357</td>
<td>780</td>
<td>96</td>
</tr>
<tr>
<td>Yeasts counts (cfu/ml)</td>
<td>1115</td>
<td>TNTC</td>
<td>114</td>
</tr>
<tr>
<td>Pseudomonas counts (cfu/ml)</td>
<td>105</td>
<td>196</td>
<td>0</td>
</tr>
<tr>
<td>E. Coli counts (cfu/ml)</td>
<td>102</td>
<td>307</td>
<td>73</td>
</tr>
<tr>
<td>Total heterotrophic bacterial counts (cfu/ml)</td>
<td>TNTC</td>
<td>TNTC</td>
<td>388</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>27.3</td>
<td>33.5</td>
<td>119</td>
</tr>
</tbody>
</table>

TNTC, Too numerous to count.

### Table 2. Mean total bacterial and fungal counts of surface water after treatment with G. Kofa seeds at one hour retention (residence) time.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Stream 1 (mile 6 Mankon)</th>
<th>Stream 2 (Bambui)</th>
<th>Water source 3 (stormwater)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coliform counts (cfu/ml)</td>
<td>92</td>
<td>215</td>
<td>9</td>
</tr>
<tr>
<td>Yeasts counts (cfu/ml)</td>
<td>43</td>
<td>507</td>
<td>48</td>
</tr>
<tr>
<td>Pseudomonas counts (cfu/ml)</td>
<td>35</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>E. Coli counts (cfu/ml)</td>
<td>32</td>
<td>55</td>
<td>13</td>
</tr>
<tr>
<td>Total heterotrophic bacterial counts (cfu/ml)</td>
<td>515</td>
<td>611</td>
<td>80</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>7.11</td>
<td>11.3</td>
<td>20</td>
</tr>
</tbody>
</table>

### Table 3. Mean total bacterial and fungal counts of surface water after treatment with Persea americana seeds at one hour retention (residence) time.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Stream 1 (mile 6 Mankon)</th>
<th>Stream 2 (Bambui)</th>
<th>Water source 3 (stormwater)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coliform counts (cfu/ml)</td>
<td>98</td>
<td>323</td>
<td>18</td>
</tr>
<tr>
<td>Yeasts counts (cfu/ml)</td>
<td>85</td>
<td>366</td>
<td>21</td>
</tr>
<tr>
<td>Pseudomonas counts (cfu/ml)</td>
<td>66</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>E. Coli counts (cfu/ml)</td>
<td>59</td>
<td>33</td>
<td>11</td>
</tr>
<tr>
<td>Total heterotrophic bacterial counts (cfu/ml)</td>
<td>823</td>
<td>516</td>
<td>95</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>13.2</td>
<td>14.11</td>
<td>42.5</td>
</tr>
</tbody>
</table>

### Table 4. Mean total bacterial and fungal counts of surface water after treatment with C. papaya seeds at one hour retention (residence) time.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Stream 1 (mile 6 Mankon)</th>
<th>Stream 2 (Bambui)</th>
<th>Water source 3 (stormwater)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coliform counts (cfu/ml)</td>
<td>82</td>
<td>296</td>
<td>13</td>
</tr>
<tr>
<td>Yeasts counts (cfu/ml)</td>
<td>12</td>
<td>59</td>
<td>19</td>
</tr>
<tr>
<td>Pseudomonas counts (cfu/ml)</td>
<td>55</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>E. Coli counts (cfu/ml)</td>
<td>63</td>
<td>37</td>
<td>18</td>
</tr>
<tr>
<td>Total heterotrophic bacterial counts (cfu/ml)</td>
<td>428</td>
<td>315</td>
<td>110</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>9.9</td>
<td>9.4</td>
<td>11.9</td>
</tr>
</tbody>
</table>
Table 5. Mean total bacterial and fungal counts of surface water after treatment with sand filter drum alone at one hour residence time.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Stream 1 (mile 6 Mankon)</th>
<th>Stream 2 (Bambui)</th>
<th>Water source 3 (stormwater)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coliform counts (cfu/ml)</td>
<td>298</td>
<td>663</td>
<td>89</td>
</tr>
<tr>
<td>Yeast counts (cfu/ml)</td>
<td>997</td>
<td>895</td>
<td>114</td>
</tr>
<tr>
<td>Pseudomonas counts (cfu/ml)</td>
<td>89</td>
<td>109</td>
<td>0</td>
</tr>
<tr>
<td>E. Coli counts (cfu/ml)</td>
<td>83</td>
<td>896</td>
<td>69</td>
</tr>
<tr>
<td>Total heterotrophic bacterial counts (cfu/ml)</td>
<td>TNTC</td>
<td>TNTC</td>
<td>279</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>25.8</td>
<td>28.91</td>
<td>95.1</td>
</tr>
</tbody>
</table>

Table 6. Mean total bacterial and fungal counts of surface water after combined treatment with G. kola seeds and sand filter drum in 1 hour residence time.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Stream 1 (mile 6 Mankon)</th>
<th>Stream 2 (Bambui)</th>
<th>Water source 3 (stormwater)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coliform counts (cfu/ml)</td>
<td>25</td>
<td>31</td>
<td>18</td>
</tr>
<tr>
<td>Yeast counts (cfu/ml)</td>
<td>39</td>
<td>69</td>
<td>31</td>
</tr>
<tr>
<td>Pseudomonas count cfu/ml</td>
<td>15</td>
<td>12</td>
<td>21</td>
</tr>
<tr>
<td>E. coli counts (cfu/ml)</td>
<td>29</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>Total heterotrophic bacterial</td>
<td>425</td>
<td>391</td>
<td>112</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>6.7</td>
<td>5.12</td>
<td>21.2</td>
</tr>
</tbody>
</table>

col counts (102,307.73 CFU/ml) and coliform counts (357,780.96 CFU/ml) shows how unsafe the surface water was in Bamenda. This may be the first report on surface water quality in Bamenda, Cameroon. The use of the stream water for bathing could expose one’s to skin diseases, especially if one has wounds or scratches on the skin. The presence and fairly high P. aeruginosa counts (105,196 CFU/ml) supports this. Pseudomonas species apart from causing urinary tract infections also causes skin infections (Cheesbrough, 1984). Surface water in Cameroon is heavily contaminated. This may be attributed to lot of human activities going on in the vicinity of these water bodies. The mean heterotrophic bacterial counts of the pond water when treated with G. kola seeds showed drastic reduction in bacterial counts from too numerous to 515,611.80 count of colony forming units per milliliter (Tables 1 and 2). However, despite a drastic reduction of E. coli and coliform counts (Tables 2, 3 and 4) of the surface water when treated with plant coagulant/disinfectants seeds, the water was still not wholesome as suggested by the values, which are higher than the WHO recommended values. This however, suggests another round of treatment, without refuting the efficiency of three plant coagulants and disinfectants. It was in consideration of this that the integration of a sand filter media to Garcia seeds treated water was carried out. When this was done, the total heterotrophic bacterial counts reduced to 425,291,112 CFU/ml. E. coli counts reduced to 29,19,21 CFU/ml, while coliform counts reduced to 25,31,18 CFU/ml. The values of heterotrophic bacterial counts were found to be in the WHO standard value for potable water (Table 6). The turbidity was drastically reduced for all the treatments (WHO, 1984) The results also indicated that the sand filter was efficient in turbidity removal (Tables 5 and 6). This is attributed to the fact that sand filter drum is made up of a number of layers of materials with different textures and filtering capacity: fine sand, coarse sand, charcoal and gravel (Yongabi et al., 2010).

Plant coagulant seeds are rich in nutrients which enter into the water and possibly serves as a substrate for bacterial re-growth (Ghebremichael et al., 2009). Water filtered through the sand filter media is stored for a longer time than with plant coagulant treated water. Despite the comparatively better water treatment potentials of the sand filter, it cannot quickly purify very dirty surface water (27,3,33.5 NTU). The sand filter drum has been very efficient in treating well water, borehole and deep stream water with low turbidity (NTU < 10 NTU).

The option of pre-disinfection with phyto-coagulants, particularly seeds of Garcinia, Carica and Perea, is not commonly practiced and require further studies. The analyses has shown that surface water can be treated effectively with plants coagulants/disinfectants and sand filter. In addition, the system may provide a greater volume of treated water at household level in a relatively shorter residence time than with just the bio sand filter system.
Conclusion

The benefits of disinfecting water with indigenous plant materials while filtering with slow sand filter drum are enormous; systems are dissolved, greater volume of treated water can be stored for a longer time, any form of dirty or wastewater can be treated and consumed within a relatively short residence time. Pathogenic microbes resistant to chlorine as well as the unpleasant effects of alum and chlorine can be taken care of without any energy inputs. The techniques are accommodating and the materials needed are cheap, and are all almost naturally available.

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http://www.loboro.ac.uk/waterresources/technical-briefs/60-waterclarification-using-moringa-olefera-seeds.p".
CHAPTER 7

INTEGRATED PHYTODISINFECTANT SAND FILTER DRUM FOR HOUSEHOLD WATER TREATMENT IN SUBSAHARAN AFRICA

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2. School of Agriculture, Food and Wine, the University of Adelaide Waite Campus

Article 4 is presented in this chapter. It follows up on the finding in Article 2. It examines the integration of plant disinfectants onto a sand filter laid in a drum to produce clean water at household level. It demonstrates the modification of the defects of the use of plant disinfectants in water. The cost effectiveness of this technology is reported in this article. Part of this article was presented in the Australian Chemical Engineering conference (Chemeca) in September 2010 in Adelaide. The paper was published in the Journal of Environmental Science and Engineering, 5 (2011) 947-954. This journal is based in the USA with a reasonable impact factor. Publication included in pages 97 – 104.

![Figure 22: Raw(L) and Moringa treated (R) water (L) and sand filter setup for household use (R)](image)

This Journal is indexed in many databases including Ulrich’s periodicals and cited more than 100 times in various journals.

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Integrated Phytodisinfectant-Sand Filter Drum for Household Water Treatment in Subsaharan Africa

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Abstract: This report details an assessment of an integrated low-technology phytodisinfectant-sand filter drum for household water and waste water treatment for sub-Saharan Africa, using bacterial culture tests, total solids, and turbidity amongst others as presented. A hundred litres of very dirty/hard water (130.3 NTU) was pretreated with 100 seeds of Moringa oleifera and further filtered through a sand filter drum (120 litres carrying capacity) made of fine, coarse sand, charcoal and gravel. The mean total aerobic mesophilic bacterial counts, E. coli, coliforms, pseudomonas, and yeast counts, as well as turbidity of untreated water drastically reduced to wealth health organization acceptable standards for potable water. The results indicated that the mean values of the same parameters for sand filtered pond water alone was significantly lower than the corresponding mean values obtained for Moringa treated pond water. The findings from this study suggest strongly that an integral of two natural water purification technologies (phytodisinfectant-sand filter drum) could be applied for the treatment of all types of contaminated water rendering it free from pathogens for potable and non-potable uses.

Key words: Phytodisinfectant, bacteria, pathogens, turbidity, sandfilter, drum, treatment.

1. Introduction

It is estimated that more than 1.2 billion people lack access to safe drinking water and close to 2.5 billion people lack adequate sanitation while 3 out of every five persons lack clean water [1]. Water remains a strategic resource for the integration of economic, social and environmental concerns and is key to sustainable development [2, 3]. It sustains human productivity and livelihoods and plays a crucial role in integrating world's ecosystems. Water is under increasing and competing demands from agricultural, industrial and domestic uses with increasing pollution threatening this scarce resource. The total volume of water as dictated by hydrological remains constant but contamination of water by geological, industrial and anthropogenic sources remains the greatest deterrent to Man's usage. About 1.6 million people are forced to use contaminated water globally [4, 5]. Uncontaminated water is rarely obtainable in rural Africa, Asia and especially in Subsaharan Africa where the prevalence of waterborne infectious diseases is sharply rising [6, 7]. Water borne diseases contribute to the death of about 4 million children in the developing countries per annum. As such, the world health organization has estimated that up to 80% of all disease and sickness in the world is caused by inadequate sanitation, polluted water or unavailability of water [3]. The need to treat waste water both for domestic use and safe disposal to the environment is obviously exigent. In most developing nations, there are legislation/legal framework established to have industries and factories treat their wastewater before disposal but the cost has been prohibitive for most companies and as such very
few companies treat their wastewater. Unlike in the Western countries, most companies treat their wastewater although at high cost, high energy inputs with complex and most often technologies that are not ecological [8]. Water scarcity remains intractable in sub-Saharan Africa, especially in rural, semi-urban and even urban areas in these countries [6, 8, 9]. Water management has become a global phenomenon. The beginning of the 21st century has experienced heightened awareness on ecological matters. Humankind has fully come to terms with the rapid urbanization and population growth that is invariably accompanied by adverse environmental problems.

There are a number of natural, social and economic activities that affect water quality and availability [3, 10]. There are some water bodies that are naturally defective due to the geology of the area. Besides, there are also natural and artificial water bodies like ponds that contain a lot of nutrients and unacceptable for consumption.

Natural disasters like the Tsunami in last December 2004 have generated a social quagmire and a scandal to the scientific world, as clean water and other embarrassing environmental challenges abound. Furthermore, conflict prone areas around Africa like the Darfur region of Sudan, DR Congo, to name a few, experience acute water crises. It has been estimated that 125 litres of water (potable) is required per person per day, yet, many households cannot actually boast of 25 litres of clean water per person per day. In tandem with this, water purification technologies would have to be reviewed in terms of its simplicity, accessibility (cost) and efficiency [4].

The search for simple, reliable and effective method of water treatment led to the use of plant materials including seeds of *Moringa oleifera* [3, 6, 11, 12]. Standard methods for the treatment of water include coagulation, flocculation, sedimentation and disinfection. These methods are often inappropriate due to prohibitive cost and low availability of chemical coagulants and disinfectants. Dosage and technique poses some local challenges, and for this reasons efforts to establish appropriate chlorination techniques for wells in rural communities is imperative [10]. Regrettably, the available technologies in present day do appear complex and still expensive. No doubt, despite tremendous awareness campaign during the water and sanitation decade of 1981-1990, water and sanitation problems today still remain a major task to reckon with. If the Millennium Development Goals in the water and sanitation sector are something to go by, then the need to focus on integrated low cost water purification systems at grassroots stands unequivocal [2, 4, 6, 8, 13].

For communities perpetually with potable water, there is a need to reflect on how the water is treated, even if the high cost is affordable; is the treatment regime in harmony with nature? Are there some side effects conceivable? Nature, though sometimes harsh is still kind and tender, maybe we fail to get her echoes. Certainly we need an “Adam and Eve water purification type of technology”.

Unfortunately, our flackery of understanding in the way natural systems function erodes our confidence and creates an absurdity about natural systems in favour of the mechanical/chemical systems. The ball has been rolling; the bucket method of water purification using *Moringa oleifera* by Folkard and Sutherland is a case in point [5, 9] although that generates less quantity of fairly pure water perhaps on a daily basis. The integration of phytodisinfectants such as moringa pretreated cum sand filtered water could provide a unique opportunity that overcomes the shortfalls of both techniques. The idea of the sand filter stems from spring water. Spring water is the purest form of water in nature as the water filters through different geological beds [8].

This study was therefore designed to investigate the potential of phytodisinfectants-sand filter system to turbid and wastewater targeted for countries in sub-Saharan Africa. The ultimate purpose is to come up with an affordable wastewater treatment technology.
that is low tech, effective and ecological. In this report, an evaluation of the potentials of integrating phytodisinfectants and phytocoagulants using *Moringa oleifera* seed powder and sand filtered treated water is presented.

2. Material and Methods

Mature seeds of *Moringa oleifera* were obtained around homes in Bauchi (Fig. 1). 100 seeds were deshelled and pulverized in clean mortar using a pestle. The powder (from 100 seeds) was sprinkled onto 100 litres of the dirty pond water in a 150 litre capacity drum (see picture) and stirred using a clean wood stirrer and the set up allowed to sit for 30 minutes. It was then filtered off using a muslin sack cloth and the filtered water was then passed through a sand filter drum [9].

The materials used in the construction of the sand filter were locally gotten at a river bed and included: low strong (150 litre carrying capacity) drum (plastic), 1 ½ yards of hose, four clips, three nipples, strainer or sieve, sharp river sand (coarse and fine), charcoal and gravel. All these materials (sand, gravel and charcoal) were carefully washed and rinsed repeatedly in clean water [5, 9].

The laying of the materials in the drum was done in the order: laying of perforated hose connected to the collector tank, then a layer of gravel, followed by a layer of charcoal, then coarse sand (2 mm in size) and two layers of fine sand (0.15-0.30 mm size) on top. A test trial was carried out by flushing the set up repeated with clean water. The moringa pretreated water was then passed through the system. The filtered water was collected in drum 2 and samples taken for analyses. Three pond water samples were collected and subjected to all these treatments.

2.1 Study Site

The pond water (surface) was located in Bauchi metropolis, Nigeria. The pond water is shallow, very turbid and during the dry season, people sometimes fetch the water for household chores, stray animals, cows drink from the source.

2.2 pH

The pH of the water samples before and after treatment with biocoagulants and sand filter was measured using a pH meter model pH 1-125. The electrodes of the pH meter were standardized by calibrating in acidic and basic buffers raised on distilled water. pH was taken by inserting the electrodes into test tubes containing wastewater samples and pH read off from the meter screen. The values obtained were consistent with values from HACH DR 2000 [14-16].

2.3 Temperature

Temperature was read off directly from the pH meter.

Total solids and turbidity were analyzed using HACH protocols. The HACH (model 44600) DR 2000 was used for the analysis. The analysis was conducted at the WATSAN laboratory, Bauchi.

2.4 Microbial Analysis and Criteria for Selection of Tests

The microbial analysis was carried out according to protocols specified by Refs. [3, 4, 17]. The MPN technique was avoided due to the fact that it is time consuming, while the membrane filter technique was not considered because of certain limitations such as lack of facility for coliform counts and total aerobic bacterial counts etc., while the presumptive coliform test is also time consuming as limited in other microbial parameters.
Chapter 7

Integrated Phytodisinfectant-Sand Filter Drum for Household Water Treatment in Subsaharan Africa

A range of tests such as total aerobic mesophilic bacterial counts, pseudomonas aeruginosa counts, as well as yeast counts were considered in addition to the traditional coliform and faecal coliform tests to explore the possibility of taking care of viable but non-culturable bacterial. Besides, the traditional indicator tests do not always correlate well with certain groups of pathogens such as Helicobacter pylori and Aeromonas spp and it is worthwhile considering this, especially in assessing the potential of newly constructed systems for water purification.

2.5 Culture Test

Three samples from a very dirty pond (surface) water with water lily growing in the water. The sample was collected using sterile Macconkey bottles [11]. 1 mL of the samples each was serially diluted with distilled water (9 mL) three fold, i.e. up to $10^{-3}$ and 1 mL of each diluent of $10^{-3}$ and $10^{-2}$ were plated aseptically onto nutrient agar for total aerobic mesophilic bacterial and pseudomonas counts, MacConkey agar for Total coliform and Eosin Ethylene Blue agar for Escherichia coli counts and potato dextrose agar for yeast/fungi counts [11, 17, 18].

The whole set ups were incubated at 37 °C for 24 hours, and plates were read off following standard microbiological procedures. The bacterial counts were enumerated using Gallemp colony counter and recorded accordingly while the yeast and pseudomonas colonies were picked, stained using methylene blue and gram stain respectively. The average counts from $10^{-3}$ and $10^{-2}$ dilutions were recorded.

3. Results and Discussion

The results are presented in Tables 1-5 and Figs. 1-3. The findings showed conclusively that combining

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Surface sample</th>
<th>water (pond)</th>
<th>Surface sample</th>
<th>water (pond)</th>
<th>Surface samples</th>
<th>water (pond)</th>
<th>Mean values (X)</th>
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<tbody>
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<td>7.5</td>
<td>7.6</td>
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<td>130.1</td>
<td>130.3</td>
<td>130.2</td>
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<td></td>
</tr>
<tr>
<td>Total aerobic mesophilic bacterial counts (cfu/mL)</td>
<td>TNLC</td>
<td>TNLC</td>
<td>TNLC</td>
<td>TNLC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>4900</td>
<td>5200</td>
<td>5100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coliform counts (cfu/mL)</td>
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<td>7300</td>
<td>7300</td>
<td>7300</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yeast counts (cfu/mL)</td>
<td>2500</td>
<td>2520</td>
<td>2338</td>
<td>2452.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas counts (cfu/mL)</td>
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<td>900</td>
<td>1118</td>
<td>1151</td>
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TNLC—Too numerous to count.

<table>
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<th>water (pond)</th>
<th>Surface sample</th>
<th>water (pond)</th>
<th>Surface samples</th>
<th>water (pond)</th>
<th>Mean values (X)</th>
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<tr>
<td>pH</td>
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<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
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<td>30.8</td>
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<td>352</td>
<td>352</td>
<td>352</td>
<td>352.0</td>
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<td></td>
<td></td>
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<tr>
<td>Total aerobic mesophilic bacterial counts (cfu/mL)</td>
<td>303*</td>
<td>305</td>
<td>352</td>
<td>352.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli counts (cfu/mL)</td>
<td>574</td>
<td>574</td>
<td>570</td>
<td>572.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coliform counts (cfu/mL)</td>
<td>438</td>
<td>439</td>
<td>436</td>
<td>437.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yeast counts (cfu/mL)</td>
<td>755</td>
<td>755</td>
<td>758</td>
<td>756.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas counts (cfu/mL)</td>
<td>156</td>
<td>151</td>
<td>155</td>
<td>154.0</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

* Larger colonies with butter-like consistency.
### Table 3: Physico-chemical and Microbial Assessment of Pond (Surface) Water After Purification with Sand Filter Drum

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Surface water (pond)</th>
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<th>Surface water (pond)</th>
<th>Surface water (pond)</th>
<th>Mean values (X)</th>
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<td>pH</td>
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<td>7.6</td>
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<td>7.6</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
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<td>22</td>
<td>22</td>
<td>22</td>
<td>22.7</td>
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<tr>
<td>Total solids (mg/dm³)</td>
<td>327</td>
<td>327</td>
<td>327</td>
<td>327</td>
<td>327</td>
</tr>
<tr>
<td>Total Aerobic Mesophilic bacterial counts (cfu/mL)</td>
<td>188</td>
<td>192</td>
<td>189</td>
<td>189</td>
<td>189.7</td>
</tr>
<tr>
<td>Escherichia coli counts (cfu/mL)</td>
<td>13</td>
<td>15</td>
<td>13</td>
<td>13</td>
<td>13.7</td>
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<tr>
<td>Coliform counts (cfu/mL)</td>
<td>15</td>
<td>17</td>
<td>15</td>
<td>15</td>
<td>15.7</td>
</tr>
<tr>
<td>Yeast counts (cfu/mL)</td>
<td>23</td>
<td>23</td>
<td>25</td>
<td>23</td>
<td>23.7</td>
</tr>
<tr>
<td>Pseudomonas counts (cfu/mL)</td>
<td>10</td>
<td>12</td>
<td>15</td>
<td>15</td>
<td>12.3</td>
</tr>
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</table>

### Table 4: Physico-chemical and Microbial Assessment of Pond (Surface) Water After a Combined Treatment with Moringa oleifera Seed Powder and Sand Filter

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Surface water (pond)</th>
<th>Surface water (pond)</th>
<th>Surface water (pond)</th>
<th>Surface water (pond)</th>
<th>Mean values (X)</th>
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<tr>
<td>pH</td>
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<td>7.6</td>
<td>7.6</td>
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<td>7.6</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>4.9</td>
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<td>4.9</td>
<td>4.8</td>
<td>4.8</td>
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<td>Total solids (mg/dm³)</td>
<td>207</td>
<td>300</td>
<td>207</td>
<td>208</td>
<td>208</td>
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<tr>
<td>Total aerobic Mesophilic bacterial counts (cfu/mL)</td>
<td>10</td>
<td>7</td>
<td>7</td>
<td>8</td>
<td>8</td>
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<tr>
<td>Escherichia coli counts (cfu/mL)</td>
<td>0</td>
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<td>0</td>
<td>0.3</td>
<td>0.3</td>
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<tr>
<td>Coliform counts (cfu/mL)</td>
<td>5</td>
<td>7</td>
<td>5</td>
<td>5.3</td>
<td>5.3</td>
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<tr>
<td>Yeast counts (cfu/mL)</td>
<td>10</td>
<td>11</td>
<td>7</td>
<td>9.3</td>
<td>9.3</td>
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<tr>
<td>Pseudomonas counts (cfu/mL)</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>3</td>
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</tbody>
</table>

### Table 5: Comparing Mean Values for Untreated, Moringa Treated, Sand Filter Treated and Moringa-sand Filtered Ponds (Surface) Water

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated pond/surface water (X)</th>
<th>Treatment Moringa oleifera (X) with Treatment sand filter drum (X)</th>
<th>Treatment sand filter drum (X)</th>
<th>WHO recommended values (ranges)</th>
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<tr>
<td>Temperature (°C)</td>
<td>27.0</td>
<td>27.0</td>
<td>27.1</td>
<td>-</td>
</tr>
<tr>
<td>pH</td>
<td>7.6</td>
<td>7.0</td>
<td>7.6</td>
<td>6.8-8.5 (0.2)</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>130.2</td>
<td>30.8</td>
<td>22.7</td>
<td>0.5 (25)</td>
</tr>
<tr>
<td>Total solids (mg/dm³)</td>
<td>466.0</td>
<td>352.0</td>
<td>327</td>
<td>200 (1000)</td>
</tr>
<tr>
<td>Total aerobic Mesophilic bacterial counts (cfu/mL)</td>
<td>394.0</td>
<td>189.7</td>
<td>8</td>
<td>0-500</td>
</tr>
<tr>
<td>Escherichia coli counts (cfu/mL)</td>
<td>5100</td>
<td>572.7</td>
<td>13.7</td>
<td>0.3 (1)</td>
</tr>
<tr>
<td>Coliform counts (cfu/mL)</td>
<td>7300</td>
<td>457.7</td>
<td>15.7</td>
<td>5.3 (10)</td>
</tr>
<tr>
<td>Yeast counts (cfu/mL)</td>
<td>2452.7</td>
<td>758.0</td>
<td>23.7</td>
<td>9.3</td>
</tr>
<tr>
<td>Pseudomonas counts (cfu/mL)</td>
<td>151</td>
<td>154.0</td>
<td>12.3</td>
<td>3 (1)</td>
</tr>
</tbody>
</table>

TNTC—Too numerous to count.
Values in brackets indicate maximum permissible limit.
**Moringa oleifera** seeds with the sand filter outfit can recover very dirty water for consumption. One moringa seed treats a litre of turbid water. Fig. 1 shows pods bearing moringa seeds. The raw data obtained from pond water showed a very high total aerobic bacterial population and high faecal indicator bacteria suggesting the presence of pathogens. This has been reported earlier by Cheesbrough [18]. Normally, surface water is not usually consumed in Subsaharan Africa but during the dry season when there is acute water shortage, people fetch such water for other non potable domestic uses. These include, laundry, washing of dishes and in some outskirts in rural places, when the situation grows deplorable, the people treat such water with Alum, boil and use it for drinking. The high *E. coli* (5100 cfu/mL) and coliform counts (7,300 cfu/mL) shows how unsafe the pond water is for use in any activity [3]. The use of the pond water for bathing could expose one to skin diseases especially if one has wounds or scratches on the skin [18]. The presence and fairly high pseudomonas aeruginosa counts [19-21] supports this. Generally, surface water in Subsaharan Africa is heavily contaminated and this holds true for most streams across Cameroon. This is as a result of a lot of human activities going on in the vicinity of these water bodies [3].

The mean bacterial counts of the pond water when treated with *Moringa oleifera* seeds showed drastic reduction in bacterial counts from too numerous to count to 394 colony forming units per millilitre. This falls within the WHO range (Table 5, Figs. 2 and 3). However, despite a drastic reduction of *E. coli* and coliform counts (Table 5) of the pond water when treated with Moringa seed powder, the water cannot still be consumed as suggested by the values, which are far off from the WHO recommended values [5, 9, 13]. This however suggests another round of treatment, without refuting the efficiency of moringa as a coagulant and disinfectant. Moreover, water treated with moringa cannot last for more 48 hours without bacterial re-growth [5, 13]. It was in consideration of this that the integration of a sand filter system to moringa treated water was carried out. When this was done, the total aerobic mesophilic bacterial counts reduced to 8 cfu/mL, *E. coli* counts reduced to 0.3 cfu/mL while coliform counts to 5.3 cfu/mL. The values were found to be in the WHO standard values for potable water (Table 5 and Figs. 1-3). The turbidity
Fig. 3 The Moringa-sand filter drum.

as well was drastically reduced within the WHO acceptable level. The results equally indicated that the sand filter is more efficient in purifying water that the seed powder of *Moringa oleifera* (Table 3). This is attributed to the fact that sand filter drum is made up of a number of layers of materials with different textures and filtering capacity, fine sand, coarse sand, charcoal and gravel.

*Moringa oleifera* is rich in nutrients which extracts into the water and later serves as a substrate for bacterial re-growth [11]. Water filtered through the sand filter stores far longer than with Moringa treated water. Despite the comparatively better water treatment potentials of the sand filter, it cannot purify very dirty pond water (the kind of water sample used in this study, Table 3) at one stretch. Without doubts, the sand filter drum has been very efficient in treating well water, borehole, and deep stream water. The CARUDEP (Catholic Arch diocesan Rural Development Programme in Jos, Plateau State of Nigeria) has been installing sand filter drums in many rural communities in Jos, Nigeria for treating the above sources of water. In cases where the water is very turbid (muddy water), they would advise the rural people to either strain the water using a sack cloth or keep in the sun for sedimentation to take place before filtering through the sand filter.

This has worked well, but the option of a pre-disinfection with phytocoagulants particularly seeds of *Moringa oleifera* is not commonly practiced as well as require further studies. It was in view of this that the need arose to evaluate the potentials of an integrated moringa/sand filter drum for purifying all sorts of available water for consumption. The analyse has shown that pond water can be treated effectively using an integrated moringa/sand filter drum. In addition, the system can provide a greater volume of treated water at household than with the Moringa bucket system reported by Folkard and Sutherland and cited by Fuglie [9].

4. Conclusion

The benefits disinfecting water with plant materials while filtering with slow sand filter drum are enormous; the short falls of the two systems are dissolved, greater volume of treated water can store longer, any form of dirty or wastewater can be treated and consumed within a relatively short time. Pathogenic microbes resistant to chlorine as well as the unpleasant effects of Alum and chlorine can be taken care of if this system is used. The techniques are accommodating and the materials needed are cheap, almost all naturally available. Studies are underway in Cameroon to scale up the system for large scale water treatment.
Chapter 7

Integrated Phyto-disinfectant-Sand Filter Drum for Household Water Treatment in Sub-Saharan Africa

References

CHAPTER 8

ALTERNATIVE PERSPECTIVES IN WATER AND WASTE WATER TREATMENT: PHYTOCOAGULANT SAND FILTER ALTERNATIVE FOR WATER AND WATER TREATMENT.

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This chapter contains Article 5, where an introduction to the research is presented. The book contains 129 pages of preliminary results on the use of plant coagulants and plant based technology in treating turbid and waste waters. The need to search for alternative and complimentary approaches to water treatment at low cost as well as lab results demonstrating the efficacy of some biocoagulants and simple designs of water treatment outfit using biocoagulants is presented. This book was published by Lambert Publishers, Ibsn 9783838387857 pp 129, Germany. The book sells for 180 USD and 80 AUSD, and is widely distributed all over the world at Amazon.com. It was the top most publication in the School of Chemical Engineering for 2010 and available digitally at digital.adelaide.edu.au (http://hdl.handle.net/2440/63838) and sold in the Engineering and Mathematic category in many countries, including the USA.

NOTE:
This figure/table/image has been removed to comply with copyright regulations. It is included in the print copy of the thesis held by the University of Adelaide Library.

Figure 23: Covers of a published book emanating from this Research work.
CHAPTER 9

NATURAL MATERIALS FOR SUSTAINABLE WATER POLLUTION MANAGEMENT

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2. School of Agriculture, food and wine, Walte Campus.

This final article in this series of six published items is presented in this chapter. This article presents an overview of water pollution management with the application of natural pollution management using natural materials – this included plant coagulants and geological materials such as geocoagulant. The term Mycocoagulants is used for the first time in literature. The results of using these materials to treat all sorts of domestic water and also their application in industrial waste waters are reported. A preliminary report on the pilot water project for a school in Cameroon using 100% ecological materials is presented. This article was published as a book chapter in Water Pollution, Issbn 9789533079622, in Croatia – Europe, with 600 downloads to date (Oct 2012). It is amongst the most cited publications in the School of Chemical Engineering for 2011/2012.

Figure 24: First ecological water treatment unit using phyto disinfectant-sand filter system
Established at a secondary school, GTC Njinikom – Cameroon supported by Phytobiotechnology Research Foundation and the University of Adelaide, South Australia.


http://hdl.handle.net/2440/71654
Natural Materials for Sustainable Water Pollution Management

Kenneth Yongabi, David Lewis and Paul Harris
School of Chemical Engineering, The University of Adelaide
South Australia

"Once you eliminate the Impossible, whatever remains, no matter how improbable, must be the truth" (Sherlock Holmes. (Sir Arthur Conan, Doyle, 1859 - 1930))

1. Introduction

The World Health Organization has estimated that up to 80% of all diseases and sicknesses in the world are caused by inadequate sanitation, polluted water or unavailability of water. (Cheesbrough, 1994; Fritchard et al., 2009 and Yongabi et al., 2010) Faeces, garbage resulting from improper sewage disposal are an important source of pathogenic organisms in water, especially the causative agents of diarrhoeal and dysentery diseases. Faeces are attractive to flies which support the development of the larval stages (maggots) of filth flies. These hazards coupled with the indiscriminate disposal of faeces can also constitute a grave nuisance from the offensive sight and smell. In many parts of rural Africa, toilets and garbage disposal pits and/or sites are cited close to wells. The leachates from these could contaminate ground and surface water (Yongabi et al., 2011) Diseases associated with water could be broadly categorised into five epidemiological groups viz: Waterborne infections e.g. cholera, typhoid, infective hepatitis. Water shortage diseases e.g. skin infections, trachoma. Water-impounding diseases e.g. schistosomiasis and guinea worm. Water-arthropod disease e.g. malaria onchocerciasis. Chemical constituents either excess or shortage e.g. fluoride - this may have an indirect effect in the body. The World population is currently growing at an unprecedented rate, and as of 1996 a 1.8% growth rate per annum which translates to over 80 million people a year was reported (UN, 1996). There is no doubt that most African countries are presently characterised by inexorable population explosion (Adegbola, 1987 and Yongabi et al., 2010) This has dire consequences to the food and environmental resources, more industries are springing up and the quest for survival is generating a lot of pollution. In order to meet the needs of this soaring population, the production capacity in all the sectors would have to be multiplied. All these sectors would need water as a raw material and apparently, there is water shortage in Sub Saharan Africa. For instance, in Nigeria, a lot of volumes of water is used and needed for irrigation especially in the dry season.

The potential irrigation area of Nigeria stands at about 2.5 Million hectares which is capable of producing close to 40% of the current total annual crop production. The small scale farmers irrigation constitutes 90% of the country's irrigation potentials (Dada et al., 1990).

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This picture is similar across sub Saharan Africa. Apart from agriculture, the health sector, hospitals and pharmaceutical companies in Nigeria use a lot of water during manufacturing and cooling systems. The waste water generated is high and often discharged untreated. This ultimately pollutes surface water. Such waste water is difficult and expensive to treat and re-use as it contains a mix of chemical compounds. The major industries in Nigeria like food-based industries and breweries and refineries really use huge volumes of water and as such generate so much waste water.

Taking the brewing industry as a good first point of call, only 8% of the nutrients in the spent grain are used. The other 92% is waste and usually discharged into the environment. Imagine 18.5 hl of wastewater (alkaline is leached out to the environment to produce a bottle of beer (Ihl) with 1.2kg of BOD, equally, the water input is high. (20hl)

Many breweries across sub saharan africa and the world over function along similar lines (Pauli, 1998) These sectors depend heavily on water which is scarce and generates a lot of wastewater that is at best untreated and goes along way to generate ecological imbalances especially in the face of population explosion. As consequence of population explosion is heavy dependence on fresh water resources, fresh water gradually becoming impoverished in many parts of the world through a number of means; contamination, reclamation and exhaustion. For instance, in Jordan and Yemen 30% of their water from their ground water aquifers are depleted per annum than the aquifers are able to recharge (Engle/Mannand /eroy, 1993). This trend may be similar across many countries the world over.

1.1 Statement of problem / justification

Estimates reveal that water borne diseases contribute to the death of 4 million children in the developing countries each year. This estimate by UNICEF may be a far under estimation of the real situation on ground. In many African countries for instance, water is scarce in both the rural and urban settings. A local survey carried out in the states of Bauchi, Plateau and Benue states shows that most of the rural communities lack potable water and has to travel many miles to search for water in nearby polluted streams for domestic uses. (Table I) At Agakwe, in Tiv Land, Edoma land in Benue state of Nigeria, pipe borne water was found (Survey done by researchers with CARUDEP, JOS, 2005) while in Bauchi, an all the local Governments in the rural areas of Tafawa Balewa, Ganiyuwa etc depend on wells that dry up in the dry season. The same holds true for many communities in the northern parts of Cameroon, Chad, Sudan, Central African Republic and Niger. The communities lack basic hygiene, sanitation and water (Table 2.) UNICEF (1993, 2009) acknowledged that the lack of universal access to health, education and water services for the world’s poorest people is a big obstacle to the global targets for sustainable development ( UNEP, 2002) Unfortunately, this obstacle remains and it is uncertain if the strategies on ground can generate sustainability in any way. This is because, poor people in semi-urban and some rural communities in sub-sharan africa still pay a disproportionate share of their meagre incomes for water services that is irregular, inconvenient and often suspicious in quality. A survey done in some villages in Cameroon shows just how potable water is rare. (Table1). Paradoxically, so much attention, the world over has been placed on water pollution and sanitation programmes with huge expenditures but the impact, however, remains questionable. Perhaps to attempt to explain this short falls could be that most of the strategies used to solve these problems are in themselves not sustainable.
<table>
<thead>
<tr>
<th>Community Name</th>
<th>No. adult male</th>
<th>No. adult women</th>
<th>Children 1-12</th>
<th>Youth 12-25</th>
<th>No. Latrines</th>
<th>No. houses</th>
<th>Water point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garin Abare</td>
<td>400</td>
<td>800</td>
<td>1500</td>
<td>700</td>
<td>120</td>
<td>300</td>
<td>5</td>
</tr>
<tr>
<td>GIKAR</td>
<td>1000</td>
<td>2250</td>
<td>4000</td>
<td>700</td>
<td>21</td>
<td>250</td>
<td>3</td>
</tr>
<tr>
<td>KWABLLANG</td>
<td>250</td>
<td>350</td>
<td>1000</td>
<td>32</td>
<td>100</td>
<td>6</td>
<td>19</td>
</tr>
<tr>
<td>Barkaya</td>
<td>500</td>
<td>600</td>
<td>3500</td>
<td>32</td>
<td>100</td>
<td>6</td>
<td>19</td>
</tr>
<tr>
<td>DUKKUN Dundima</td>
<td>25</td>
<td>36</td>
<td>59</td>
<td>18</td>
<td>32</td>
<td>13</td>
<td>19</td>
</tr>
<tr>
<td>Turiya</td>
<td>25</td>
<td>38</td>
<td>88</td>
<td>15</td>
<td>15</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Nassarawa</td>
<td>35</td>
<td>42</td>
<td>81</td>
<td>19</td>
<td>26</td>
<td>29</td>
<td>5</td>
</tr>
<tr>
<td>Dindima</td>
<td>25</td>
<td>36</td>
<td>56</td>
<td>19</td>
<td>38</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>Fumbinare</td>
<td>42</td>
<td>59</td>
<td>102</td>
<td>54</td>
<td>49</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>G/Total</td>
<td>2302</td>
<td>4211</td>
<td>10386</td>
<td>1625</td>
<td>365</td>
<td>828</td>
<td>73</td>
</tr>
</tbody>
</table>

*Done in collaboration with Development Exchange Centre (DEC), an NGO which provides / assist these communities with development projects in 2005*

Table 1. Baseline survey on water and sanitation facilities from communities in Bauchi state, Nigeria (The survey indicates limited safe water and sanitation among the rural area in Bauchi-Nigeria)

<table>
<thead>
<tr>
<th>Illnesses</th>
<th>Adults No%</th>
<th>Children No%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scabies</td>
<td>24 (2.9%)</td>
<td>32 (3.8%)</td>
</tr>
<tr>
<td>Skin sepsis</td>
<td>16 (1.9%)</td>
<td>20 (2.4%)</td>
</tr>
<tr>
<td>Yaws</td>
<td>4 (0.5%)</td>
<td>10 (1.2%)</td>
</tr>
<tr>
<td>Lice</td>
<td>152 (18.3%)</td>
<td>250 (30.0%)</td>
</tr>
<tr>
<td>Trachoma</td>
<td>16 (1.9%)</td>
<td>18 (2.2%)</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>65 (7.8%)</td>
<td>133 (16.0%)</td>
</tr>
<tr>
<td>Bacillary dysentery</td>
<td>106 (12.7%)</td>
<td>198 (23.8%)</td>
</tr>
<tr>
<td>Salmonellosis</td>
<td>16 (1.9%)</td>
<td>28 (3.4%)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>14 (1.7%)</td>
<td>108 (13.0%)</td>
</tr>
<tr>
<td>Ascariasis</td>
<td>10 (1.2%)</td>
<td>18 (2.2%)</td>
</tr>
<tr>
<td>Paratyphoid Fever</td>
<td>20 (2.4%)</td>
<td>34 (4.1%)</td>
</tr>
<tr>
<td>Worms</td>
<td>4 (0.5%)</td>
<td>24 (2.9%)</td>
</tr>
<tr>
<td>Stomach ache</td>
<td>-</td>
<td>103 (2.9%)</td>
</tr>
<tr>
<td>Malaria</td>
<td>70 (8.4%)</td>
<td>6 (0.7%)</td>
</tr>
<tr>
<td></td>
<td>517 (53.7)</td>
<td>986 (117.4%)</td>
</tr>
</tbody>
</table>

Table 2. Frequency distribution of common illnesses found in Bulli Village, 10km away from the university Community in Bauchi, Nigeria. (The results in this table indicates high frequency of water borne diseases in the study area)

Providing pipe borne water to communities is laudable but when the low income earning communities cannot cope with maintenance cost and the robustness of the technologies in place, then it becomes a major problem. UNICEF (1993) reported that in the 1980s, some 10 million dollars was spent yearly in the developing countries on high technology to improve services to people who already had water and sanitation predominantly in the cities. Only a fraction (20%) was reluctantly spared on low-cost appropriate technology.

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for the underserved majority of people in peri-urban areas (UNICEF, 1993). The high cost of treating water and its attending high energy input is prohibitive to most industries and factories in developing countries and as such release untreated wastewater into neighbouring streams, thereby polluting many fresh water bodies. These sources of water pollution includes heavy metals, halogenated hydrocarbons, dioxins, organochlorines such as DDT which do not easily break down under natural processes and tend to accumulate in biological food chain. The popular treatment system of water in sub sahara africa is the sedimentation, coagulation, disinfection (chlorination), filtration. Undoubtedly, this has generated potable water but, however, the final water products remain unaffordable by 70% of the populace (Schultz et al., 1988; Yongabi et al., 2010) Reports also suggest chlorine resistant organisms such as, cryptosporidium oocysts, strains of salmonella sp, aeromonas, entamoeba cyst, mycobacterium sp, escherichia Coli. 0157:47 and host of others (Madore et al., 1987; Yongabi et al., 2011). Chlorine has been noted as a potential carcinogen forming compounds such as tetrachloromethylene (TCM) which also produces hormonal analogue that may interfere with male fertility. Aluminium sulphate (Alum), the widely used water coagulant thus generate acidic water, unsafe for pregnant women and causes predementia in some people (loss of memory) While all these defects exists, mankind has been endowed with indigenous knowledge and has been using it to survive proceeding the advent of all these technologies. There is a need to revisit our roots, study this indigenous system and improve on them. Exploring and exploiting the potentials of natural materials such as plants and sand to bring about cheap clean water in a more ecological friendly manner are the thrust of this work. This may have great lessons for ecological sustainability now and centuries to come.

1.2 Aims / objectives
The ultimate purpose of this chapter is to report results of our research on a water pollution management technology that is low-tech, cheap and above all ecologically friendly. The specific objectives of this study, therefore, are: to report the results of analysis of the pathogen level of polluted water from refinery, food and confectionery processing industry in Nigeria and Cameroon, stagnant pond water where people fetch water for household chores and for irrigation at. To carry out a survey / inventory on problems of clean water and indigenous knowledge on how communities treat their water in Nigeria and Cameroon. To use the collected knowledge and screen these plant materials and their extract for their coagulation/ disinfection activities in vitro using polluted water samples. To test their potential antimicrobial activity on isolates from polluted water samples and, to generate clean water using a constructed integrated biocoagulant - sand filter system and other geological-materials.

2. Brief overview of interdisciplinary importance, dangers of water and existing gaps in water pollution management

2.1 The necessity of water as a consumable product in all the aspects of life
The role of water in life as a whole cannot be over emphasised as this universal solvent is the basis of life after air. What a life without water? Evolutionary, biologists hold strongly that life began in water and therefore explains why human use water at times for rituals. Water is a prime necessity for life, it forms the basis for a balanced diet without which digestion cannot function well. It is a lubricant for biological processes such as

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excretion and major glands secretion are usually in water form. It acts as a cushion preventing crushing in internal structures, example synovial fluid. To the agriculturalist, crops cannot grow without water, therefore, it is needed for germination, that is probably why in dry areas irrigation is used to shunt this adversity. Water is used for laundry, domestication such as cooking and washing of utensils. This vital community is the basis for electricity in which case more important than electricity. It is the source for hydroelectricity which is the backbone of all industries and factories. Additionally, water is used in agro-industry for washing, media for dissolution, production of dairy products, beverages etc. To the engineer, water is used as a cooling agent, lubricant and for building and construction. In addition, water is a useful transport source: Navigation. Water is a habitat for fish and minerals such as petroleum. Fish being used as sources of protein for man and petroleum and other minerals used as fuels.

Water serves as a touristic site, for example the Knobi beach in Cameroon and the Gubi dam in Bauchi state. In the wise, a source of revenue for the Government. Indeed, highlighting the use of water could only tantamount to an infinite list. Taking water as previously mentioned is a source of a balanced diet, it contains vital minerals such as Manessium, Ca, Fe, Cu, Zu, F, No, So, etc) for the International Standard for drinking water (WHO, 1984). Therefore, good water should actually posses these minerals. Good water should be colourless, odourless and free from any toxic elements. Some toxic substances in drinking water could include Pb, Se, As, Cr and CN. According to World Health Organization, 1988, showed that a 0.001 ppm is the maximum concentrations allowable, exceeding this level is pollution (see As cited above, toxic elements could be consumed from water that could lead to cancer. Water, even though has many uses serves as a breeding ground for some vectors of man's parasitic diseases, for example, Malaria and schistosomiasis. Rainwater in excess could cause flood and hence heavy economic losses. Besides, this could also lead to erosion which inflicts heavy pains to Agriculture. Finally, sea accidents lead to loss of lives too. The overwhelming indispensability of water as a primordial stuff for all the aims of Economy has become a hot topic for discussion by many state governors and their administrations in Africa. If the State governments are not supplying boreholes and other rural water Sources, she is either trying to solve flood problems or some other hazard cause by rain storm. From analysis of all budgets speeches since 1982 to date, it is interesting to note that water has been placed as a top priority amongst other projects yet little is achieved. Water which is safe for drinking must be free of pathogenic organisms, toxic substances and an excess of minerals and organic debris. It must be colourless, tasteless and odourless in order to be attractive to consumers and preferably cool. Water is the basis of life. About 75% of the body weight is made of water. In developing countries 15 million infants die every year due to contaminated drinking water, poor hygiene and malnutrition. About 80% of illness in developing countries are directly connected with contaminated drinking water (WHO). The Provision of water supply near by for consumers and sufficient for their daily needs will help greatly in decreasing the incidence of skin diseases and eye infections and also reduce diarrhea diseases and most worm infections, particularly if the water is of good quality bacteriological. However, major improvements in health conditions through provision of sufficient safe water can only be achieved through domestic hygiene and proper methods of water purification (Yongabi et al. 2010). Oyawaye et al. (2000) in their study of water sources for three years in Bauchi, noted elevated levels of nitrates (33.3mg/kg) in ground water.
sources in the dry season. Higher nitrate values for treated and untreated waters still remained high in the rainy season but within acceptable limits. Excess Nitrate in water has been linked to methemoglobinemia (bleu babies). Many infant deaths in Africa and particularly sub-Saharan Africa are mainly attributed to dysentery and diarrhoea of undefined sources which may be due to nitrates in water. Similarly, literature elsewhere has evidence that implicates N-Nitrosamines in the incidence of carcinogenesis. The density of Microbial isolates has been reported to be inversely proportional to the level of residual chlorine from 1.0mg/L to less than 0.2mg/L. Residual chlorine also reduces steadily from point of application to point of collection. Twenty three bacterial genera belonging to groups of coliform, faecal coliform and Staphylococcus spp were isolated at various stages (Yongabi et al., 2011).

2.2 General methods of water pollution management

2.2.1 Application of chlorine, halogens and alum in water treatment

Chlorine is widely applied to disinfect water. For instance, a well or a tank containing 1000 litres of relatively clean water 2g of chlorine is added and if organic matter is present or one is doubting the purity you add 4g of chlorine. Then thoroughly mix it into the water and allow standing for at least 30 minutes before using it. However if water is highly turbid i.e containing a lot of sediments, alum is first added to make the sediments settle at the base. The water is then drain into another tank before chlorinating. The amount of Alum required treating 1000 litres of relatively clean water is 5g while for sufficient safe water for a community but then it requires highly skilled technicians who can measure and control the chlorine and alum dosage. This knowledge is lacking in the rural areas. Other halogens such as bromine and iodine are also applied in water treatment. The set backs have been discussed in recent publications (Yongabi et al., 2010 and Yongabi et al., 2011).

2.2.2 Sand filter

This method of purifying water has been known right from time immemorial. Over thousands of years now clean water have been obtained from river beds when dug. As water falls or flows over the river bed it percolates through the sand grains where the disease-causing organism filter out. Clean Sharp River sand is obtained and thoroughly washed; gravels are also obtained and washed. Trow clean containers are used for the construction of the sand filter. The container for the filter and storage could be made out of metal plastic or traditional clay. A hole is made two-thirds of the way up the filter container and hose with blocked base and perforated will be fixed at the opening into the drum. The gravel is then placed over it to a height of 7.5cm and the sand is placed above it to a height just below the hose fitting. The filter is then thoroughly flushed out with clean water for a week to allow for formation of biofilm. The attributes and set backs of the sand filter in terms of cost, installation, management and efficacy and the need to intergrade it with plant coagulants have been reported (Yongabi et al., 2010).

2.2.3 Water treatment with plants: The case of moringa oleifera and water hyacinth

The seed pods of Moringa oleifera have been used for water treatment. After shelving, the seeds are crushed, seized (3.5mm mesh) using traditional techniques employed in the production of maize flour. Approximately 50-150mg of the ground seed will be needed to

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treat a litre of river water, depending on the quantity of suspended matter. Normally, a small amount of clean water is then mixed with the crushed seed to form a paste. The crushed seed powder when added to water, yields water soluble proteins that possess a net positive charge. The coagulant/flocculant characteristics of seed is linked to a series of low molecular weight cationic protein. Dose of Moringa oleifera seeds depends much on the turbidity of the water in question. Generally, 75-250mg/l (.75 - 2.5g) has been employed. For a turbidity of 400NTU, 1.5g of Moringa oleifera powder is used for a litre of turbid water. Extensive studies have been done on the applications of Moringa oleifera in water treatment. Other plants used in water treatment include, cactus, water hyacinth and spondrachus potatorum which are reported to remove turbidity and heavy metals from water (Bima, 1991; Yongabi et al., 2010) The need to catalogue such useful natural materials in Africa needs intensification.

3. Materials and methods

3.1 Study area
The study was conducted in Bauchi State (at Abubakar Tafawa Balewa University) Nigeria and Bamenda, Cameroon 2010 to 2011. These two countries are located in sub saharan Africa with the same climatic conditions and similar traditions and problems. These Natural plant materials collected from these focussed more on other plants rather than Moringa oleifera which has been extensively reported in literature.

3.2.1 Materials used
MacCathney and bijore bottles were purchased from supplies of Hospital and laboratory materials from bauchi metropolis. They were washed repeatedly using detergent and rinsed in clean water and then sterilised by autoclaving alongside with all glasswares used for the study. Autoclaving was done at 121°C for 15 minutes.
A number of agars; Nutrient agar (oxoid Ltd), MacConkey Eosine Methylene blue, potato Dextrose agars (Oxoid) Ltd were obtained from the University Zeri Research laboratory and Phytobiotechnology Research laboratory and School of Chemical engineering, The University of Adelaide, South Australia.

3.2.2 Special equipment and apparatus
Some of the equipment and apparatuses used for the study include spectrophotometer (Phips) Vu/Vis, Pve, unicon sp 6-450 incubator (jouan) bench. Centrifuge (Mistrail 1000) weighing balance (meter am100) and soxhlet apparatus (Galler kamp).

3.2.3 Chemicals and reagents
Nutrient Agar were obtained from biotech laboratories survey, UK. Ferric chloride, potassium hydroxide, copper acetate, lead acetate, bismuth nitrate sodium chloride, chloroform, diethylether, ethanol were purchased from Britiest Drug Houses (BDH). Chemicals Ltd, poole England.
Ammonia solution potassium tartrate picric acid, philing solutions were obtained from Maud B Ltd England.
All other reagents an chemicals used were of analytical grade obtained from reputable scientific and chemical companies. All solutions were prepared in distilled water, redistilled from pyrex apparatus.

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Carica Papaya Plant
seeds used as a phytodisinfectant and coagulant in rural Cameroon

Clay
used as a geocoagulant

Pieces of Alum  Garcinia Kola seeds
Alum is a synthetic coagulant used as a phytocoagulant and phytodisinfectant, widely used, people have to buy. locally available in the communities in Africa
3.2.4 Polluted water sample collection
Refinery wastewater was collected from other Kaduna Petroleum oil refinery. This is located in kaduna Town in Kaduna State in the northern parts of Nigeria and SONARA oil in Cameroon. The crude oil is fractionated and fuel produced amongst other, bye products. The refinery wastewater is usually discharged untreated into river Kaduna. Ten litres of the wastewater was collected with the assistance of students undertaking their internship at the refinery in 2005-2011. Wastewater from the NASCO Company Ltd in Jos, plateau State of Nigeria was also collected. The NASCO household in Jos produces a number of confectioneries including; biscuits, cornflakes etc and then Nasco soaps, detergents etc. Jos is located in the North Central region of Nigeria and has a teeming population. The wastewater is usually discharged into the neighbouring streams and brooks. Ten litres of the wastewater was collected with the acid of students on internship. Lastly, dirty (turbid) pond water was collected from a stagnant pond located at the western part of the Abubakar Tafawa Balewa University Campus. The stagnant water is used by the local people around for irrigation of Crops within the vicinity, other activities include fishing and washing of clothes as well as at times swimming. Ten litres of the sample was collected for laboratory studies.

3.3 Microbiology analyses
One ml of each of the samples was diluted in 9ml of sterile distilled water and serially diluted up to 10-5 dilution and plated in triplicates on Nutrient agar for total heterotrophic bacterial counts, MacConkey agar and Eosine Methylene blue agars for Total Coliform and E. coli counts respectively while on potato dextrose agar for fungal counts. Incubation was done at 37°C for 24 hours for bacterial counts and at 25°C for fungal counts. Discrete colonies on each plates were counted on each plate and average of three plates taken. The presence of colonies on Eosine methylene blue agar indicated probable identify for typical coliform colonies. Gram stained portions of the colonies showed gram negative rods with absence of spores as a further elucidation of the Micromorphology of coliform. Colonies on EMB that appeared as Metallic greenish sheen confirmed the presence of Escherichia Coli.

3.3.1 Collection and identification of plants
The leaves and Ahus precatorius were collected from Shere hills, Jos Nigeria. This plant sample was authenticated by plant taxonomists at the Federal College of forestry Jos.

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Nigeria. They were dried and pounded into well labeled, clean air tight. Containers and stored until required.

### 3.3.2 Sources of test organisms
Clinical isolated of *Escherichia coli*, *staphylococcus aureus*, *Salmonella paratyphi* and *Candida albican* were isolated from polluted streams in Bamenda, Cameroon.

### 3.3.3 Methods
#### Preparation of plants for biological test
The dried and pulverized samples were then extracted using ethanol, diethyl and water. This was done in increasing polarity. Most traditional healers sometimes use palm wine, which contains ethanol as their solvent. This gives additional reason for preferring ethanol to methanol. Diethyl ether is one of the best solvent for antimicrobial activities (nastro et al, 2000). Water is a universal solvent.

#### 3.3.4 Aqueous extraction
10g of powdered sample was weighed on a Mettle balance. It was then put in separate clean and sterile conical flasks containing 200ml of cold sterile.

#### 3.3.5 Biological assay
#### Preparation of dilutions of the extracts
The concentrations of various crude extracts were made in sterile distilled water and for (diethyl ether extract) the concentrations prepared in those solvents were 50ml, 100mg/ml and 150mg/ml.

#### 3.3.6 Purification of bacterial isolates
The stock cultures of the bacterial isolates were subculture unto nutrient agar, blood agar and maccoukey agar to produce discrete colonies and incubated for 24 hours at 37°C. The plates were examined for purity and specific biochemical test were carried out to confirm the identity of the different isolates according to methods described by (Baker et al, 1980).

#### 3.3.7 Test for bacterial suspensions
Preparation of fresh plates of the test bacteria was made from isolated stocks stored on agar slants. By the use of a stride wire loop, colonies of fresh cultures were picked and suspended in 20ml of nutrient broth in different sterile universal bottles. The centrifugation bottles were done in MSE refrigerating centrifuge at 1000m rpm for 30 minutes in virology department of N.V.R.I Vom. The supernatant was discarded. The organisms were again resuspended using equal volumes of sterile normal saline. The concentrations of the organism were obtained by comparison with 10 standard opacity bottles (Macfarland's Naphelometry) method of opacity, which contained various amounts of barium sulphate in 1% sulphheric acid (N/36). Most of the tubes corresponded to 10^-4 which was very turbid. The organisms were then diluted down to 10^-6 s then one looped equivalent of 0.02ml from each of the bottles 10^-4, 10^-5 and 10^-6 was plated out on three different
petridishes containing nutrient agar and incubator over-right at 37°C to determine population density of the test organism. Member of colony forming units per milliliter was obtained as follows, since for example 10^8 dilution had 25 colonies. Colonies on the second day 25 x 50 x 10^6 + 1.25 x 10^9 C.FU/ml. 1ml of the 10^-6 dilution of various bacteria was used in flooding nutrient agar plates in the agar diffusion method of invitro sensitivity test.

3.3.8 Preparation of media
2.5g of nutrient agar was dissolved in 100ml of distilled water and heated slowly while shaking until the solution become clear and yellow in colour. The nutrient agar was cool to about 47°C and become semi-solid state. This is to facilitate the diffusion of large molecules of the crude extract as compound or standard or processed and purified antibiotics with small and readily diffusible molecules.

3.4.1 Agar gel diffusion test (punch-hole method)
The plates of nutrient agar were seeded in duplicates with 1.0ml of 10^-6 dilution of the test bacteria. The plates were then swirled to allow the inoculum to spread on the excess was discarded in a disinfectant jar. The plates were allowed on the bench for 5 minute is and they were dried in the incubator for 1 hour at 37°C.
Using a sterile cork borer four well were bored at equal distances around for plate. The 5th well was made in the middle. The bottoms of the wells were sealed with one drop each of sterile nutrient agar before the extracts were put.
The prepared concentrations the extracts were put into the wells. Sterile distilled water was put in the 5th well to serve as negative control for aqueous and ethanolic extracts while dimethylsulfoxide ws used as negative control for diethylether. Gentamycin was used as a positive control in the 4th well. After allowing on the the bench for 1 hour, for diffusion of the extracts, the plates were incubated at 37°C for one day. The plates were examined the next day to concentrations of the extracts on the test bacteria.
The zones of inhibitions were measured using a ruler in millimeters and the average of the two readings was taken to be the zone of inhibition of the bacterial species in a particular concentration.

3.4.2 Minimum Inhibitory Concentration (MIC)
This was determined using broth dilution technique (Puyelde, 1956). Freshly prepared broth in sterile Bijou bottles was used. Two sets of six Bijou bottles were used for each test. 1 ml of sterile nutrient broth was put in Bijou bottle number 1 to 6.1ml 200mg/ml was added to Bijou bottle number one. The extract in the bottle on was therefore diluted 1:2. It was properly mixed and 1ml was transferred to bottle number two which was diluted 1:4 and this was continued until the 5th bottle from which one ml was discarded. Bottle number six contained only sterile nutrient broth to serve as negative control.A loopful of 10^-6 dilution of bacteria suspension with microbial load of 1.25 x 10^9 C.F.U/ml was then added to all the six bottles. This entire procedure was done for all the organisms that were susceptible to the various extracts. The bottles were thoroughly mixed by gentle shaking and incubation for 24 hours at 37°C. The bottles were observed for turbidity after incubation visually by comparing with the control. Cultures from incubated bottles were subcultured onto fresh nutrient agar plates. The inoculated were incubated at 37°C for 24 hours. The plates were

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examined for growth indicated bacteriocidal effect of the concentration of the extract used. Plates showing light growth were taken to have bacteriostatic effect, while those showing moderate or heavy growth were taken to have no inhibitory effect on the bacteria (Puyuelde, 1986).

3.4.3 PH analysis
The PH of the raw and treated wastewater samples was tested using a combi-9 test strip (a standard strip for routine urinary biochemical analysis). A fresh strip each was dipped into each of the samples and after sixty seconds, the colour change noticed was compared with a range of colour standards and when the colour of the strip matched any of the colour standards, the PH label was directly read off.

(Photo field solar weighing balance and Combi-9 Ph strip)

3.4.4 Turbidity evaluation
A subjective visual observation was done. The presence of colloidal suspended matter was noted in the untreated samples while their absence noted in the treated samples. Floc formation and lack of floc formation was also observed as a distinct evidence of coagulation for the treated samples. The presence of odour and absences was also noted by snuffing the nose. The use of the sight and small senses were highly exploited.

3.4.5 Plant sample selection and collection
The plant coaguants used in this study were selected based on a survey of their local use in water purification by the indigenous people in sub-saharan africa (Yongabi K. A. 2004, www.biotech.kth.se/jobb/new/kenneth04.doc) Moringa Oleifera (Lam) seeds have been used by a rural Nigeria for water treatment and literature elsewhere abound (Fuglie, 1999, Folkland et al. 2000.) The dried seed of Moringa Oleifera were harvested from Bauchi State, Nigeria. Seeds of Garcinia Kola, Hibiscus sabdarifla and Carica papaya were collected from Enugu in Nigeria and Bamenda, Cameroon (Photo).

3.4.6 Plant processing
The seed pods were harvested and stored in Khaki envelopes, They were deshelled (specifically M Oleifera Garcinia Kola) while the seed of Carica papaya were scooped out from riped fruits as well as Hibiscus sabdarifla seeds were purchased from the market at Mudulawal Market in Bauchi Metropolis.

3.4.7 Coagulation studies
Graded weight (0.5g to 5g) of the pulverized plant Materials each and Alum, Hydrogen peroxide, were each added to 200mls of each of the wastewater samples in 250ml capacity beakers.

Increased weights in grams from 0.5g to 5.0g of each of the plant material was mixed in a small quantity of turbid water for form a paste and then mixed carefully with the water samples in the beakers.

The same procedure was done for Alum and a turbid water sample in a beaker (200mls. was allowed to stand in a beaker for 24 hours as controls). The Coagulative effects and change in total bacterial counts, PH, visual clarity amongst other parameters were evaluated.

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3.5.1 Cold extraction (Buck extraction)
A cold Methanol and aqueous Extraction was then carried out on 50 grams each of Hibiscus sabdariffa seed and Carica papaya seed powders except for Moringa Oleifera. 50 grams of each of the powders was steeped in 250mls each of methanol and water for 24 hours. Gravity filtration was carried out using whatman filter paper No 13 and solvent evaporated at room temperature.

3.5.2 A cold sequential extraction of Moringa oleifera and Garcina kola seeds
A cold Sequential solvent Extraction was carried out on Moringa oleifera seed powder using n-hexane, Dichloromethane Methanol and water in that order. The purpose of this was to exploit the polarity effect of the solvent on the possible isolation of the active portion from the plant material. 50grams of the pulverized seed (pulverised using a pestle and mortar) was steeped in 250ml of n-hexane left for 24 hours, filter off using gravity filtration using whatman filter paper No 13, The plant residue was dried in the sun and used for the next solvent and the order maintained for all the other solvents. The extracts were left in the open for 2 weeks for the solvent to evaporate. The extracts were now used for antibacterial bioassay.

3.5.3 Antibacterial assay (agar diffusion method)
The bacterial isolates were re-cultured in peptone water for 18 hours and 0.3ml of each of the bacterial suspension was mixed aseptically with 15ml nutrient agar (oxoid) in sterile petri plates and allowed to solidify. A stainless steel borer of 6mm diameter was used to punch wells into the agar and each well was filled with 0.1ml of 2% extract, and with oil and of sterile distilled water, H2O2 and Alum as controls.

3.5.4 Phytochemical screening
The phytochemical screening of the powdered extracts obtained from the leaves of Abrus precatorius were carried out using standard qualitative procedures (Trelease and Evans 1989, Sofowora 1986).

3.5.5 Test for alkaloids
Two grams of plants materials thoroughly grounded was treated in a test tube with 25ml of 1% Ad for 15min in a water bath. The suspension wasfiltrated in a test tube and the filtrate was divided in two parts A and b.
To filtrate A, five drops of Dragendorff reagent were added. The formation of a precipitate indicated the presence of alkaloids.

3.5.6 Test for flavonoids
i. Well ground plant material (1g) was extracted with water (10ml) and methanol (5ml) and filtered. Few magnesium turnings were added to 3ml of filtrate and concentrated added dropwise (cyanidine reaction). Developments of colour indicate the presence of flavonoids a red colour and flavonones give a pink colour.
ii. To 1ml of the extract 1ml of Naoh was added. The formation of a golden yellow precipitate indicated the presence of flavonoids.

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3.5.7 Test for cardiac glycosides (salkowski test)
0.5g of extract was added to 2ml of chloroform and after mixing, 2ml of H2O were carefully added to from a lower layer. reddish brown colour at the interface indicates the presence of a steroidal ring i.e. glycogen portion of the cardiac glycoside.

3.5.8 Test for anthraquinones
Anthraquinones are a subset of anthranoids. For the specific test an ether chloroform maceration (1g in 5ml of CH3 and 5ml of ethered was filtered and 1ml of 10% NaOH solution. A red quinones. A weak coloration was assigned a +, while a strong coloration a +++.

3.5.9 Test for steroids
Powdered plant material (1g) was covered with ether and shaken occasionally for 2 hours. The solution was filtered and decanted. 1ml of the solution was put on porcelain plate to evaporate. A drop of conc. H2SO4 was added and stirred orange coloration was positive indication.

3.6.1 Test for saponins
Well-grounded plant material (1g) in water (15ml) in a test tube was heated on water bath for 5 minutes. The solution was filtered and left to cool to room temperature. The filtrate (10ml) in 16 x 100mm test tube was shaken for 10 seconds and the height of honeycomb trough, which persisted, was measured. Froth higher than 1cm confirms the presence of saponins.

3.6.2 Test for tannins
10ml of water were added to 5g of extract ad the mixture was stirred and filtered. To 2ml of the filtered. To 2ml of the filtrate few drops of 0.1% FeCl3 solution and the development of precipitate was observed. A blue-black, green precipitate indicates the presence of tannins.

3.6.3 Test for carbohydrate
5g of the powder sample was boiled in 10ml distilled water on hot plate for 5 minutes and filtered while hot. The filtrate was used for the following tests.

i. Molisch test
To 3.0ml of the 2 filtrate was added 3 drops of molisch reagents then carefully run 3.0ml conc. H2SO4 without shaking. The interphase formed was then observed for purple.

ii. Benedict's test
3 drops of the filtrate was added to 2.0ml of benedict reagent and placed on a hot plate for 5 minutes to observe the formation of brick red precipitate

3.6.4 Balsam test
To 3 drops of alcoholic ferric chloride was added to 2.0ml of extract then warm a dark green coloration if formed with balsam. To 2.0ml of the extract were added few drops of potassium permanganate. The solutions was then warmed on hot plate and observe for benzaldehyde or almond adour.
This was carried out in duplicate, and each set up was incubated at 37°C for 24 hours and the diameter of zone of inhibition in mm was recorded using a vernier caliper.

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3.6.5 Phytochemical screening
3.6.6 Test for alkaloid
Twenty (20) mg of each of the extract was placed into a test tube, 1ml of distilled water and 2 drops of 1% HCL were added and the solution was warmed gently in a water bath to effect complete dissolution of the extract. A stream of dragendorff’s reagent was added to the solution from a test tube.

3.6.7 Test for glycosides
A ml (10) of each of the extract solution was placed in a test tube and a drop each of 2% 3,5 dinitrobenzoic acid in Methanol and 5% OH in water was added.

3.6.8 Test for tannins
A ml (1) of each of the extract was placed in a test tube and a stream of 5% FeCl₃ solution was added.

3.6.9 Test for flavonoids
Twenty (20) mg of each of the extract was dissolved in 2ml ethanol in a test tube, a small size spatula full of zinc powder was added and a few drops of HCL was then added.

3.7.1 Test for soluble carbohydrates
Twenty (20) mg of each of the extract was dissolved in 1ml distilled water and 2 drops of 5% L-nathol solution in methanol added in a test tube. While holding the tube at an angle, a stream of cone H₂SO₄ was added to it.

3.7.2 Test for saponin
Twenty (20) mg of each of the extracts was dissolved in 1ml of distilled water and 2 drops of 1% HCl was then heated gently on a water bath.

3.8 Construction of a sand filter
The design of a sand filter using two 200 litres plastic drums. The drum is cleaned out and hole is made two thirds of the way up so that an outlet pipe can be filtered. Depending on the size of the nipple, the hole is made. The water-collecting pipe is made with of hose piping. This is connected to the outlet pipe by a short hose piping. A number of saw cuts of drilled hole are made in hose piping ring and this is laid down on the bottom of the drum. The second drum is constructed for a storage drum. First a hole is made at the same level as that on the first drum and an appropriate nipple is fitted. A connecting hose is fix from the filter to the hole on the storage drum. Another hole is made at the other side and at the bottom of the drum at a height of about 7cm from the base and a water collecting pipe if fitted such that it is long enough to be dipped at the top. In other cases a tap could be fitted for collecting water, but this can easily become loose as a result of constant opening and closing so the hose is more preferable.
For setting up of the filter, clean sharp river sand of different sizes are obtained form a riverbed and sieved out. Gravels and coal of the correct sizes are also obtained and thoroughly washed with clean water; the sand is also thoroughly washed too kept in a place safe from dirt and dust.
3.8.1 Sand/gravel filter

This was constructed using sand and gravel only as media. The gravels are first placed at the bottom to a height of 75mm (7.5cm), then followed by a layer of coarse sand to a height about 100mm. The last layer of fine sand is placed to a height just below the level of outlet pipe. This arrangement is made so that even if the tap is left on, the water drains out of the filter, a small layer of water remains above the sand. The sand must never be allowed to go dry, otherwise the biologically active ingredients in the sand which are important to the purification process, will die out. Both drums should have a lid to cover the drum, and this is made with a sieve or strainer for water with allot of sediments. The sieve should be covered too.

When the filter has been completed, it must be thoroughly flushed through with clean water to further remove any dirt present. Once this is completed, a daily routine of adding raw water is maintained for a week or more so that the filter skin can form before usage begins. The design of the working components of the filter using a 200 litre drum should provide at least 624 litre of water per day. The yield rate will be controlled or regulated to 0.4 litres/minutes because the rate of flow of the filtered water to be slow to ensure satisfactory performance. However, if the raw water to be filtered has a bad odour, taste or colour, layer of coal can be introduced between the sand and the gravel layers to control the situation. The procedure outlined above is for a 200 litre drum but the same technique can be used for a brick built container, metal drum, or clay pots. Below are some models of sand filter using different media and each had the calculation of the yield rate as a guide for other types of containers.

4. Results highlights

The picture below shows the turbidity clearance level with various treatments including untreated storm water left as control.

Storm water being treated with Alum in 15 minutes, water appears clear. 1 storm water treated with Garcinia kola seeds, particles settle comparable to Alum. The third treatment container from left is storm water treated with Hibiscus seeds, particles settle but not as clear as Alum and Garcinia. The fourth treatment container from left is untreated storm water left as control, less settlement of particles.

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Chapter 9

Natural Materials for Sustainable Water Pollution Management

The findings are presented in the following tables. Data in Table 1a shows the pH and bacterial counts of foul wastewater from refinery, in the untreated wastewater, the total bacterial and fungal counts were high with a strong foul odour. Pseudomonas was also isolated in the untreated wastewater. In Table 1b, after treatment with plant materials, the total microbial counts dropped significantly to tolerable levels, the pH was stabilized while odour was no longer perceived. Pseudomonas spp was no more isolated. However, the various degree of treatment varies with the different plant materials applied. Moringa oleifera, Garcinia kola and Carica papaya exhibited the best results.

<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>Colour</th>
<th>PH</th>
<th>Smell</th>
<th>Appearance</th>
<th>THBC CF ml</th>
<th>Coliforms CF ml</th>
<th>E. Coli CF ml</th>
<th>TFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated OVH</td>
<td>Colourless</td>
<td>6.6</td>
<td>Engine oil</td>
<td>Clear</td>
<td>560</td>
<td>Nil</td>
<td>Nil</td>
<td>315</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>crude</td>
<td>Oil smell</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated DFH</td>
<td>Brownish</td>
<td>7.05</td>
<td>strong</td>
<td>Turbid</td>
<td>300</td>
<td>nil</td>
<td>nil</td>
<td>6140</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Engine oil</td>
<td>Oil smell</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* OVH --> Overhead fraction of foul wastewater  * Pseudomonas spp isolated
* DFH --> Desalter foul water

Table 3. (a) Effect of plant seed powders and Alum on oil Refinery wastewater from Kaduna State, Nigeria (Significant reduction in turbidity and microbial load using plant coagulants and disinfectants as indicated in the table)

<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>Colour</th>
<th>PH</th>
<th>Smell</th>
<th>Appearance</th>
<th>THBC CF ml</th>
<th>Coliforms CF ml</th>
<th>Coli CF ml</th>
<th>TFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>(b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated OVH</td>
<td>Colourless</td>
<td>6.6</td>
<td>Engine oil</td>
<td>Clear</td>
<td>560</td>
<td>Nil</td>
<td>Nil</td>
<td>315</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>crude</td>
<td>Oil smell</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment with</td>
<td>Very</td>
<td>7.0</td>
<td>odour absent</td>
<td>very clear</td>
<td>36</td>
<td>Nil</td>
<td>Nil</td>
<td>100</td>
</tr>
<tr>
<td>Moringa Oleifera</td>
<td>colourless</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>seed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment with</td>
<td>Very</td>
<td>7.0</td>
<td>odour absent</td>
<td>Clear</td>
<td>70</td>
<td>Nil</td>
<td>Nil</td>
<td>173</td>
</tr>
<tr>
<td>Garcinia Kola</td>
<td>Colourless</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>seed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment with</td>
<td>Colourless</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carica Papaya</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>seeds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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| Treatment with Hibiscus sabdariffa seeds | Colourless | 5.0 | odour (faint) | Clear | 133 | Nil | Nil | 113 |
| Treatment with Alum | Very Colours | 5.0 | Odour (faint) | Very clear | 313 | Nil | Nil | 6140 |

(c)

| Untreated DFH | Brownish | 7.05 | strong engine oil smell | Turbid | 300 | Nil | Nil | 6140 |
| Treatment with Moringa oleifera seed | Very clear colourless | 7.0 | odour absent | Clear | 96 | Nil | Nil | 89 |
| Treatment with Garcinia Kola Seeds | Clear colourless | 7.0 | no treatment with | Clear | 89 | Nil | Nil | 125 |
| Alum | Clear (very) | 5.0 | odour persist faintly | Clear | 122 |Nil | Nil | 168 |

In table 4 below, the results indicated that plant materials exhibited great disinfection potentials on grey water (detergent based water) when compared to alum.

<table>
<thead>
<tr>
<th>Types of treatments</th>
<th>THBC (Cfu/ml)</th>
<th>Coliforms (Cfu/ml)</th>
<th>E Coli (Cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated waste water sample</td>
<td>2,200</td>
<td>2,300</td>
<td>1,900</td>
</tr>
<tr>
<td>Untreated waste water sample left on bench and analysed</td>
<td>2,120</td>
<td>2,224</td>
<td>1,892</td>
</tr>
<tr>
<td>Alum treated sample</td>
<td>600</td>
<td>1,070</td>
<td>600</td>
</tr>
<tr>
<td>Moringa Oleifera treated sample</td>
<td>320</td>
<td>520</td>
<td>343</td>
</tr>
<tr>
<td>Jatropha Curcas treated sample</td>
<td>770</td>
<td>890</td>
<td>729</td>
</tr>
<tr>
<td>Garcinia Kola treated sample</td>
<td>700</td>
<td>675</td>
<td>521</td>
</tr>
<tr>
<td>Carica papaya treated sample</td>
<td>697</td>
<td>682</td>
<td>575</td>
</tr>
<tr>
<td>Persia americana treated sample</td>
<td>800</td>
<td>760</td>
<td>690</td>
</tr>
<tr>
<td>Hibiscus sabdariffa treated sample</td>
<td>600</td>
<td>800</td>
<td></td>
</tr>
</tbody>
</table>

* Wastewater normally stored for a week and then disposed 5g of powders of plant seeds used.

Table 4. Effects of Plant seed powders and alum on grey water detergent based water from Nasco Factory Jos, Nigeria

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Table 5, the data shows that the physicochemical properties of the detergent based water such as turbidity and pH was significantly reduced when treated with the plant based coagulants when compared with the untreated wastewater sample.

<table>
<thead>
<tr>
<th>Treatment Material</th>
<th>Turbidity assessment</th>
<th>PH</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated wastewater sample</td>
<td>Very turbid, foamy blush</td>
<td>6.5</td>
<td>Odour intense turbidity remains the same</td>
</tr>
<tr>
<td>Untreated wastewater sample left on bench and analysed</td>
<td>Remains turbid, foamy</td>
<td>6.5</td>
<td>Odour intense turbidity the same</td>
</tr>
<tr>
<td>Alum treated sample</td>
<td>Fast precipitation, very clear, slight odour</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Moringa Oleifera treated sample</td>
<td>Flocs formed, odour totally removed</td>
<td>7.0</td>
<td>Odour removal colour removal protein positive</td>
</tr>
<tr>
<td>Jatropha Curcas treated sample</td>
<td>Flocs formed settled at bottom</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>Garcinia Kola treated sample</td>
<td>Clear, flocs formed with a suspended pellet</td>
<td>6.0</td>
<td>Second stage treatment clearer, odour off, after proper filtration</td>
</tr>
<tr>
<td>Curica papaya treated sample</td>
<td></td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>Persoon americana treated sample</td>
<td>clear, no odour flocs settled at the bottom</td>
<td>6.0</td>
<td>a second stage treatment was better</td>
</tr>
<tr>
<td>Hibiscus sabdariffa seeds treated sample</td>
<td>Flocs settled at the bottom must slight odour</td>
<td>6.0</td>
<td>Protein positive</td>
</tr>
</tbody>
</table>

Table 5. Physicochemical properties of treated and untreated detergent based wastewater from food/detergent factory

In table 6, the data shows that the plant seed powders demonstrated a significant disinfection properties on stagnant water frequently used for irrigation, more than Alum. This observation has been extensively reported for Moringa (Yongabi et al., 2010) but not with the other plant materials used in this study.

<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>THBC Cfu/ml</th>
<th>Coliform counts Cfu/ml</th>
<th>E Coli counts Cfu/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated water sample initially collected</td>
<td>TNTC</td>
<td>TNTC</td>
<td>8,900</td>
</tr>
<tr>
<td>Untreated water Sample left to settle and supernatant analysed</td>
<td>TNTC</td>
<td>TNTC</td>
<td>7,900</td>
</tr>
<tr>
<td>Alum treated</td>
<td>3,598</td>
<td>1,380</td>
<td>980</td>
</tr>
<tr>
<td>Moringa Oleifera seed treated</td>
<td>485</td>
<td>298</td>
<td>125</td>
</tr>
<tr>
<td>Jatropha seeds treated</td>
<td>2,212</td>
<td>598</td>
<td>386</td>
</tr>
<tr>
<td>Garcinia Kola treated</td>
<td>387</td>
<td>452</td>
<td>294</td>
</tr>
<tr>
<td>Curica papaya seed treated</td>
<td>868</td>
<td>483</td>
<td>223</td>
</tr>
<tr>
<td>Persoon americana seed treated</td>
<td>1,201</td>
<td>822</td>
<td>429</td>
</tr>
<tr>
<td>Hibiscus sabdariffa seed treated</td>
<td>258</td>
<td>205</td>
<td>110</td>
</tr>
</tbody>
</table>

* TNTC – Cfu/mL>10,000

Table 6. Effect of plant seed powders and Alum on stagnant water from a dirty pond and used for irrigation of crops at Abubakar Tafawa Balawa University.

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In Table 7, data indicates significant changes in pH and turbidity when plant materials are applied in a 24-72 hours retention time.

<table>
<thead>
<tr>
<th>Treatment materials</th>
<th>Turbidity assessment</th>
<th>pH range</th>
<th>Other Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated water sample initially collected</td>
<td>No floc formed at all</td>
<td>7.0</td>
<td>bad odour perceived</td>
</tr>
<tr>
<td>Untreated water sample left to settle and supernatant analysed</td>
<td>Few particles stuck to the wall of the container</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td>Alum treated</td>
<td>Floc formed and settled</td>
<td>5.0</td>
<td>Odour (faint)</td>
</tr>
<tr>
<td>Moringa Oleifera treated</td>
<td>Floc settled and good settlement</td>
<td>7.0</td>
<td>Very clear and second stage treatment very good, odour no trace.</td>
</tr>
<tr>
<td>Jatropha seed treated</td>
<td>Floc formed particles settle</td>
<td>7.0</td>
<td>Odour of Jatropha</td>
</tr>
<tr>
<td>Garcinia Kola treated</td>
<td>Floc settled with suspended pellicle</td>
<td>7.0</td>
<td>Water clear and a second stage treatment clearer no odour.</td>
</tr>
<tr>
<td>Carica papaya seed treated</td>
<td>Floc formed slowly</td>
<td>7.0</td>
<td>papaya odour</td>
</tr>
<tr>
<td>Persia americana seed treated</td>
<td>Floc settlement seen slowly</td>
<td>7.0</td>
<td>no dour</td>
</tr>
<tr>
<td>Hibiscus sabdariffa seed treated</td>
<td>Floc settled at the bottom</td>
<td>5.0</td>
<td>Little odour</td>
</tr>
</tbody>
</table>

Table 7. Physicochemical properties of Treated and Untreated stagnant water used for irrigation of crops at Abubakar Tafawa Balewa University.

In Table 8, the results show a significant level of disinfection of storm water by the plant materials in comparison with Alum. This findings is in tandem with a similar findings using Hibiscus, Moringa and Jatropha by Yongabi et al., 2011

<table>
<thead>
<tr>
<th>Types of Treatment</th>
<th>THBC CF/ml</th>
<th>Coliform CF/ml</th>
<th>E Coli CF/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated storm water sample initially collected</td>
<td>TNTC</td>
<td>TNTC</td>
<td>TNTC</td>
</tr>
<tr>
<td>Untreated storm water sample left to settle and supernatant analysed (24hours)</td>
<td>9,280</td>
<td>212</td>
<td>36</td>
</tr>
<tr>
<td>Alum treated storm water</td>
<td>9,280</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Jatropha seeds treated water</td>
<td>6,930</td>
<td>180</td>
<td>20</td>
</tr>
<tr>
<td>Moringa Oleifera seeds treated storm water</td>
<td>120</td>
<td>40</td>
<td>11</td>
</tr>
<tr>
<td>Garcinia Kola seeds treated water</td>
<td>6,33</td>
<td>160</td>
<td>18</td>
</tr>
<tr>
<td>Carica Papaya seeds</td>
<td>398</td>
<td>29</td>
<td>5</td>
</tr>
<tr>
<td>Persia americana seeds</td>
<td>5,360</td>
<td>64</td>
<td>0</td>
</tr>
<tr>
<td>Hibiscus sabdariffa</td>
<td>4,024</td>
<td>50</td>
<td>32</td>
</tr>
</tbody>
</table>

* 1. Storm water harvested in flowing through the dirty streets of Yelwa after heavy rains.
   2. 5g of each the seed powder Alum into 100mls of the wastewater and left on bench for 24 hours.

Table 8. Effect of plant seed powders and Alum on Storm water collected from Bauchi Metropolis.

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Not only did the microbial content changed after treatment with the natural materials but the pH and turbidity also changed considerably as shown in the data in table 9.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Materials</th>
<th>Turbidity assessment</th>
<th>pH range</th>
<th>Other remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated storm water sample initially collected</td>
<td>No floc formed, no settlement of particles, brownish and dusty smell</td>
<td>6.9 - 7.0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Untreated storm water sample left on bench for 24 hours</td>
<td>No floc formed, few particles settle at the bottom, supernatant still has suspended particles. Some stuck to the walls of the container.</td>
<td>6.9 - 7.0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Alum treated storm water</td>
<td>Floc formed and fast, water clear supernatant clear</td>
<td>5.0</td>
<td>Clean and clear standard coagulant (1st)*</td>
<td></td>
</tr>
<tr>
<td>Moringa Oleifera seeds treated storm water</td>
<td>Floc formed when seeds dispersed in water, floc settled slowly supernatant</td>
<td>7.0</td>
<td>Moringa mildly extracts in water, Good Coagulant (2nd)*</td>
<td></td>
</tr>
<tr>
<td>Jatropha Cucanas seeds treated</td>
<td>Floc formed gently and settle</td>
<td>7.0</td>
<td>Good Coagulant (4th)*</td>
<td></td>
</tr>
<tr>
<td>Garcinia Kola seed</td>
<td>Floc formed, particles settled, very good coagulant at the bottom Excellent</td>
<td>6.9-7.0</td>
<td>Good Coagulant (3rd)*</td>
<td></td>
</tr>
<tr>
<td>Carica papaya seed</td>
<td>Floc formed, particles settled</td>
<td>6.9-7.0</td>
<td>Good Coagulant (6th)*</td>
<td></td>
</tr>
<tr>
<td>Persea americana</td>
<td>Not very clear excellent, particles</td>
<td>6.9-7.0</td>
<td>not a good coagulant (5th)*</td>
<td></td>
</tr>
<tr>
<td>Hibiscus sabdariffa</td>
<td>Excellent, particles settled</td>
<td>5.0-5.0</td>
<td>Good Coagulant (5th)*</td>
<td></td>
</tr>
</tbody>
</table>

Table 9. Physicochemical properties of treated and untreated storm water with Alum and plant seed powders.

In the table 10 below, combined plant material with clay was applied in the treatment of refinery wastewater, this hybrid plant and geological material significantly improved the water quality bacteriologically and physicochemically than with the application of just either of the materials alone.

<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>Colour</th>
<th>PH Smell</th>
<th>Appearance</th>
<th>THBC</th>
<th>Coliform</th>
<th>Ecoli</th>
<th>TFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated OVH</td>
<td>colourless</td>
<td>6.6 Engine oil, crude oil smell</td>
<td>Clear</td>
<td>560</td>
<td>Nil</td>
<td>Nil</td>
<td>315</td>
</tr>
<tr>
<td>Treatment with Moringa Oleifera seeds</td>
<td>Very clear no odour</td>
<td>7.0 no Odour</td>
<td>clear</td>
<td>36</td>
<td>Nil</td>
<td>Nil</td>
<td>100</td>
</tr>
<tr>
<td>Treatment with clay</td>
<td>clear</td>
<td>7.0 no odour</td>
<td>clear</td>
<td>32</td>
<td>nil</td>
<td>nil</td>
<td>96</td>
</tr>
<tr>
<td>Untreated DFH</td>
<td>Brownish</td>
<td>7.05 Strong engine oil smell</td>
<td>Turbid</td>
<td>300</td>
<td>nil</td>
<td>nil</td>
<td>6140</td>
</tr>
<tr>
<td>Treatment with Moringa Oleifera seeds</td>
<td>very clear no odour</td>
<td>7.0 Odour absent</td>
<td>clear</td>
<td>96</td>
<td>nil</td>
<td>nil</td>
<td>89</td>
</tr>
<tr>
<td>Treatment with clay powder</td>
<td>Clear</td>
<td>7.0 Odour absent</td>
<td>clear</td>
<td>93</td>
<td>Nil</td>
<td>Nil</td>
<td>84</td>
</tr>
</tbody>
</table>

Table 10. Effects of Combined Moringa Oleifera seed powder and clay in the treatment of oil refinery wastewater

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In the table 11 below, a combined plant material comprising plants (moringa oleifera seed powder) and sand filter media was applied to treat refinery wastewater and the results indicated a significant improvement in water quality both bacteriologically and physicochemically better than with either of the materials alone. This corroborates a similar observation using surface water in Cameroon (Yongabi et al., 2010 and Yongabi et al., 2011).

<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>Colour</th>
<th>PH</th>
<th>Smell</th>
<th>Appearance</th>
<th>THBC</th>
<th>Coliform</th>
<th>Ecoli</th>
<th>TFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated OVH</td>
<td>Colourless</td>
<td>6.6</td>
<td>Engine oil, crude oil smell</td>
<td>Clear</td>
<td>560</td>
<td>Nil</td>
<td>Nil</td>
<td>315</td>
</tr>
<tr>
<td>Treatment with Moringa Oleifera seeds powder</td>
<td>Very Colourless</td>
<td>7.0</td>
<td>Odour absent</td>
<td>very clear</td>
<td>36</td>
<td>Nil</td>
<td>Nil</td>
<td>100</td>
</tr>
<tr>
<td>Final treatment with sand</td>
<td>Colourless</td>
<td>7.0</td>
<td>Odour absent</td>
<td>Very clear</td>
<td>3</td>
<td>nil</td>
<td>nil</td>
<td>nil</td>
</tr>
<tr>
<td>Untreated DFH</td>
<td>Brownish</td>
<td>7.05</td>
<td>Strong engine oil smell</td>
<td>Turbid</td>
<td>300</td>
<td>nil</td>
<td>nil</td>
<td>6140</td>
</tr>
<tr>
<td>Treatment with Moringa Oleifera seeds powder</td>
<td>Very clear no colour</td>
<td>7.0</td>
<td>Odour absent</td>
<td>clear</td>
<td>96</td>
<td>nil</td>
<td>nil</td>
<td>89</td>
</tr>
<tr>
<td>Final treatment with sand</td>
<td>Very clear</td>
<td>7.0</td>
<td>Odour absent</td>
<td>clear</td>
<td>10</td>
<td>nil</td>
<td>nil</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 11. Effect of combined Moringa Oleifera seed Powder and sand filter media on oil refinery waste water

The data in table 12 below also shows similar findings using Garcinia kola sand filter media as with moringa oleifera sand filter media.

<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>Colour</th>
<th>PH</th>
<th>Smell</th>
<th>Appearance</th>
<th>THBC</th>
<th>Coliform</th>
<th>Ecoli</th>
<th>TFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated OVH</td>
<td>Colourless</td>
<td>6.6</td>
<td>Engine oil, crude oil smell</td>
<td>Clear</td>
<td>560</td>
<td>Nil</td>
<td>Nil</td>
<td>315</td>
</tr>
<tr>
<td>Treatment with Garcinia Kola seeds powder</td>
<td>Very Colourless</td>
<td>7.0</td>
<td>Odour absent</td>
<td>Clear</td>
<td>70</td>
<td>Nil</td>
<td>Nil</td>
<td>173</td>
</tr>
<tr>
<td>Final treatment with sand</td>
<td>Colourless</td>
<td>7.0</td>
<td>Odour absent</td>
<td>clear</td>
<td>15</td>
<td>nil</td>
<td>nil</td>
<td>8</td>
</tr>
<tr>
<td>Untreated DFH</td>
<td>Brownish</td>
<td>7.05</td>
<td>Strong engine oil smell</td>
<td>Turbid</td>
<td>300</td>
<td>nil</td>
<td>nil</td>
<td>6140</td>
</tr>
<tr>
<td>Treatment with Garcinia Kola seed powder</td>
<td>Clear very little odour</td>
<td>7.0</td>
<td>Odour clear</td>
<td>89</td>
<td>nil</td>
<td>nil</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>Final treatment with sand</td>
<td>Clear no colour</td>
<td>7.0</td>
<td>Odour clear</td>
<td>13</td>
<td>nil</td>
<td>nil</td>
<td>29</td>
<td></td>
</tr>
</tbody>
</table>

Table 12. Effects of Combined Garcinia Kola seed powder and sand filter media on oil refinery wastewater

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In tables 13, 14, 15, 16 and 17 the combined performance of the plant materials and clay, and cobined plant materials and sand filtered on various polluted water samples was tested bacteriologically and physicochemically. The results generally indicated strongly that these natural materials have strong ability to purify any type of water. The materials alone have the ability to treat water and wastewater but the combined effect of these materials have an added advantage in treating all kinds of polluted water as demonstrated by the data in the following tables 13, 14, 15, 16 and 17.

<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>Colour</th>
<th>PH</th>
<th>Smell</th>
<th>Appearance</th>
<th>THBC</th>
<th>Coliform</th>
<th>Ecoli</th>
<th>TFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated waste water sample (detergent)</td>
<td>Bluish dirty</td>
<td>6.5</td>
<td>acid</td>
<td>Turbid and foamy</td>
<td>2,200</td>
<td>2,300</td>
<td>1,900</td>
<td>-</td>
</tr>
<tr>
<td>Treatments with Moringa seed powder</td>
<td>Blue colour fades away</td>
<td>7.0</td>
<td>odour absent</td>
<td>flocs formed</td>
<td>320</td>
<td>520</td>
<td>343</td>
<td>-</td>
</tr>
<tr>
<td>Treatment with clay</td>
<td>Clear</td>
<td>7.0</td>
<td>odour absent</td>
<td>clear needs filtration</td>
<td>309</td>
<td>511</td>
<td>338</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 13. Effects of Moringa Oleifera seed powder and clay in the treatment of detergent based waste

<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>Colour</th>
<th>PH</th>
<th>Smell</th>
<th>Appearance</th>
<th>THBC</th>
<th>Coliform</th>
<th>Ecoli</th>
<th>TFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated OVH</td>
<td>colourless</td>
<td>6.6</td>
<td>Engine oil, crude oil smell</td>
<td>Clear</td>
<td>560</td>
<td>Nil</td>
<td>Nil</td>
<td>315</td>
</tr>
<tr>
<td>Treatment with Hibiscus Salutaris seed powder</td>
<td>Colourless</td>
<td>5.0</td>
<td>odour faint</td>
<td>clear</td>
<td>133</td>
<td>nil</td>
<td>nil</td>
<td>113</td>
</tr>
<tr>
<td>Treatment with sand filter media</td>
<td>very colourless</td>
<td>5.0</td>
<td>total odour removal</td>
<td>very clear</td>
<td>5</td>
<td>nil</td>
<td>nil</td>
<td>15</td>
</tr>
<tr>
<td>Untreated DFH</td>
<td>Brownish</td>
<td>7.05</td>
<td>Strong engine oil smell</td>
<td>Turbid</td>
<td>300</td>
<td>nil</td>
<td>nil</td>
<td>6140</td>
</tr>
<tr>
<td>Treatment with Hibiscus Salutaris seeds</td>
<td>clear</td>
<td>5.0</td>
<td>little odour</td>
<td>clear</td>
<td>118</td>
<td>nil</td>
<td>nil</td>
<td>595</td>
</tr>
<tr>
<td>Treatment with sand filter media</td>
<td>clear</td>
<td>5.0</td>
<td>No odour</td>
<td>clear</td>
<td>3</td>
<td>nil</td>
<td>nil</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 14. Effects of Combined Hibiscus sabdariffa seed powder and sand filter media on oil refinery wastewater

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<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>Colour</th>
<th>PH</th>
<th>Smell</th>
<th>Appearance THBC</th>
<th>Coliform</th>
<th>Ecoli</th>
<th>TFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated OVH</td>
<td>colourless</td>
<td>6.6</td>
<td>Engine oil, crude oil smell</td>
<td>Clear</td>
<td>560</td>
<td>nil</td>
<td>315</td>
</tr>
<tr>
<td>Treatment with carica papaya seeds</td>
<td>Colourless</td>
<td>7.0</td>
<td>odour absent (papaya scent)</td>
<td>Clear</td>
<td>62</td>
<td>nil</td>
<td>87</td>
</tr>
<tr>
<td>Treatment with sand filter media</td>
<td>very colourless</td>
<td>7.0</td>
<td>odour absent</td>
<td>very clear</td>
<td>2</td>
<td>nil</td>
<td>5</td>
</tr>
<tr>
<td>Untreated DFH</td>
<td>Brownish</td>
<td>7.05</td>
<td>Strong engine oil smell</td>
<td>Turbid</td>
<td>300</td>
<td>nil</td>
<td>6140</td>
</tr>
<tr>
<td>Treatment with Carica papaya seeds</td>
<td>Clear very little odour</td>
<td>7.0</td>
<td>Little odour</td>
<td>clear</td>
<td>90</td>
<td>nil</td>
<td>95</td>
</tr>
<tr>
<td>Treatment with sand filter media</td>
<td>Clear</td>
<td>7.0</td>
<td>no odour</td>
<td>clear</td>
<td>nil</td>
<td>nil</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 15. Effects of Combined Carica papaya seed powder and sand filter media on oil refinery

<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>Colour</th>
<th>PH</th>
<th>Smell</th>
<th>Appearance THBC</th>
<th>Coliform</th>
<th>Ecoli</th>
<th>TFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated waste water sample (detergent)</td>
<td>blush dirty</td>
<td>6.5</td>
<td>acid smell</td>
<td>Turbid and foamy</td>
<td>2,200</td>
<td>2,300</td>
<td>1,900</td>
</tr>
<tr>
<td>Treatment with Moringa Oleifera powder seed</td>
<td>flocs formed colour removed</td>
<td>7.0</td>
<td>odour removed totally</td>
<td>clear, no foams</td>
<td>320</td>
<td>1,070</td>
<td>343</td>
</tr>
<tr>
<td>Treatment with sand filter media</td>
<td>clear</td>
<td>7.0</td>
<td>no odour</td>
<td>clear</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 16. Effects of Combined Moringa Oleifera seed powder and sand filter media on detergent based

<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>Colour</th>
<th>PH</th>
<th>Smell</th>
<th>Appearance THBC</th>
<th>Coliform</th>
<th>Ecoli</th>
<th>TFC</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated wastewater sample (detergent)</td>
<td>very foamy blush</td>
<td>6.5</td>
<td>acid smell</td>
<td>Turbid foamy</td>
<td>2,200</td>
<td>2,300</td>
<td>1,900</td>
</tr>
<tr>
<td>Treatment with Garcinia Kola seed powder</td>
<td>flocs formed with a suspended pellicle</td>
<td>7.0</td>
<td>smell reduced</td>
<td>becoming clear</td>
<td>700</td>
<td>675</td>
<td>521</td>
</tr>
<tr>
<td>Final treatment with sand filter media</td>
<td>clear</td>
<td>7.0</td>
<td>odour absent</td>
<td>clear</td>
<td>100</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 17. Effects of Combined Garcinia Kola seed powder and sand filter media on detergent based water

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In Table 18 below, one of the plants, Moringa oleifera, was used to study its effect on unicellular organisms in water. The results indicated that Moringa oleifera seed powder gets rid of unicellular organisms such as amoeba, microalgae such as Spirogyra from water. The need to study the application of plant materials in the removal of microalgae from water systems could be rewarding.

<table>
<thead>
<tr>
<th>Types of Organisms</th>
<th>approximate number per field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diatoms</td>
<td>up to 15 per field</td>
</tr>
<tr>
<td>Cercaria</td>
<td>a few</td>
</tr>
<tr>
<td>Euglena</td>
<td>35 cells per field, actively motiles</td>
</tr>
<tr>
<td>Cyclops</td>
<td>a few</td>
</tr>
<tr>
<td>Amoeba</td>
<td>More than 15 per field</td>
</tr>
<tr>
<td>Debris</td>
<td>a lot of debris</td>
</tr>
<tr>
<td>Spirogyra</td>
<td>a lot</td>
</tr>
</tbody>
</table>

a) Microscopy of pond/stagnant water before treatment

<table>
<thead>
<tr>
<th>Types of Organism</th>
<th>approximate number per field</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euglena</td>
<td>totally absent, water clean</td>
</tr>
<tr>
<td>Diatoms</td>
<td>absent</td>
</tr>
<tr>
<td>Spirogyra (blue/green algae)</td>
<td>absent</td>
</tr>
<tr>
<td>Cyclops and cercaria</td>
<td>absent</td>
</tr>
<tr>
<td>Amoeba</td>
<td>absent</td>
</tr>
</tbody>
</table>

b) Microscopy of Pond/Stagnant water after treatment with Moringa Oleifera seed powder, and after filtration

Table 18. Effects of Moringa Oleifera seed powder on free living organisms in pond water used for irrigation

An novel attempt was made to classify materials that can be applied in water pollution management and shown in Table 19 below. More studies for a detailed classification are underway.

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<table>
<thead>
<tr>
<th>Botanical name of plants</th>
<th>Common name/ Hausa name</th>
<th>Part used</th>
<th>Types of wastewater</th>
<th>Types of Coagulant</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sychonos Potatoto</td>
<td>-</td>
<td>Seeds</td>
<td>Domestic water</td>
<td>Phyto- Coagulant</td>
<td>--------</td>
</tr>
<tr>
<td>Calotropis procera</td>
<td>Tumfafiya</td>
<td>Intox</td>
<td>Industrial wastewater</td>
<td></td>
<td>Pers. comm.</td>
</tr>
<tr>
<td>Citrus aurantiifolia</td>
<td>Limes, lemu</td>
<td>Seeds</td>
<td>Domestic water</td>
<td></td>
<td>--------</td>
</tr>
<tr>
<td>Pumice</td>
<td>Rock</td>
<td>-</td>
<td>domestic and industrial wastewater</td>
<td>Geocoagulant</td>
<td>Internet</td>
</tr>
<tr>
<td>Bentonite</td>
<td>Rock</td>
<td>-</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>Immansil</td>
<td>&quot;</td>
<td>-</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

Table 19. Survey and classification of Natural materials for water pollution management in local communities

In table 20, the nature of extracts from Garcinia kola was described. The water extract is a black solid. The coagulant and disinfection activity of Garcinia kola observed in this study may be soluble in water. More studies are need in this dimension.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Boiling point</th>
<th>Volume of Solvent (ml)</th>
<th>Nature of Extract</th>
<th>Crude yield (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichloromethane</td>
<td>-</td>
<td>-</td>
<td>Golden yellow oily semi solid</td>
<td>-</td>
</tr>
<tr>
<td>N-Hexane</td>
<td>69°C</td>
<td>250</td>
<td>Golden yellow oily semi solid</td>
<td>0.90</td>
</tr>
<tr>
<td>Toluene</td>
<td>III°C</td>
<td>250</td>
<td>Dark yellow oily semi solid</td>
<td>2.10</td>
</tr>
<tr>
<td>Acetone</td>
<td>56°C</td>
<td>250</td>
<td>Dark semi solid</td>
<td>1.90</td>
</tr>
<tr>
<td>Methanol</td>
<td>65°C</td>
<td>250</td>
<td>Dark brown solid</td>
<td>1.70</td>
</tr>
<tr>
<td>Water</td>
<td>100°C</td>
<td>250</td>
<td>Black solid</td>
<td>1.10</td>
</tr>
</tbody>
</table>

Table 20. Analysis of Phytochemical tests on solvent extracts of Garcinia Kola

The results in table 19 gives an attempt to classify some of the phytoconstituents in this plant materials. More phytonutrients were detected in the aqueous extract suggesting an easy and cheap means of extracting water treatment chemicals from Garcinia kola.

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Chapter 9

Natural Materials for Sustainable Water Pollution Management

<table>
<thead>
<tr>
<th>Solvent Extract</th>
<th>Cardiac Glycosides</th>
<th>Saponin</th>
<th>C_{6}H_{12}O_{6}</th>
<th>Tannins</th>
<th>Flavonoids</th>
<th>Alkaloids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichloromethane</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Toluene</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acetone</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Methanol</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Water</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 21. Result of Preliminary phytochemical analysis of Solvent Extracts of Garcinia Kola

The data in table 22 shows that plant materials can significantly stabilize pH of various polluted water. This has been observed with moringa in previous studies (Yongabi et al., 2010) but has not been done using various wastewater samples such as from cement and asbestos.

<table>
<thead>
<tr>
<th>Types of water/waste water</th>
<th>PH (Normal)</th>
<th>PH (treated)</th>
<th>PH (treated)</th>
<th>PH (treated)</th>
<th>PH (treated)</th>
<th>PH (treated)</th>
<th>PH (treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dirty tap water</td>
<td>6.62</td>
<td>5.0</td>
<td>7.0</td>
<td>6.99</td>
<td>5.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Yelwa tap water</td>
<td>7.36</td>
<td>5</td>
<td>5.0</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>University tap water</td>
<td>7.25</td>
<td>5.0</td>
<td>7.0</td>
<td>7.0</td>
<td>5.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Yelwa well water</td>
<td>7.37</td>
<td>5.0</td>
<td>7.0</td>
<td>7.0</td>
<td>5.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Asbestos water (well)</td>
<td>7.46</td>
<td>5.0</td>
<td>7.0</td>
<td>7.0</td>
<td>5.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Asbestos tap water</td>
<td>7.53</td>
<td>5.0</td>
<td>7.0</td>
<td>7.0</td>
<td>5.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Cement waste water</td>
<td>8.01</td>
<td>5.0</td>
<td>7.0</td>
<td>7.0</td>
<td>5.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Cement waste water</td>
<td>8.52</td>
<td>5.0</td>
<td>7.0</td>
<td>7.0</td>
<td>5.0</td>
<td>7.0</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Table 22. PH Content of various waste water/water treated with Alum and plant seed powders

To further demonstrate the disinfection potential of the plant materials on the wastewater samples, a methanol extract of the plant materials was conceivable. The resulting extracts were tested on various bacterial isolates from all the polluted water samples. The data in table 21 below demonstrates a significant level of antibacterial activity comparable to Alum.

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Table 23. Effect of cold Methanol and Aqueous Extract of Garcinia Kola, Carica papaya and Hibiscus Sabdariffa seeds on Bacterial isolates from waste water (Diameter zone of inhibition in mm)

<table>
<thead>
<tr>
<th>Extracts</th>
<th>E coli</th>
<th>Pseudomonas Sp</th>
<th>Klebsiella Sp</th>
<th>Staphylococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garcinia Kola seeds Aqueous</td>
<td>60mm</td>
<td>6mm</td>
<td>15mm</td>
<td>18mm</td>
</tr>
<tr>
<td>Extract Methanol Extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hibiscus sabdariffa seeds</td>
<td>10.8mm</td>
<td>12.0mm</td>
<td>12mm</td>
<td>15mm</td>
</tr>
<tr>
<td>Aqueous Extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methanol Extract</td>
<td>11mm</td>
<td>11.8mm</td>
<td>8mm</td>
<td>19mm</td>
</tr>
<tr>
<td>Carica papaya Seeds Aqueous</td>
<td>9mm</td>
<td>12mm</td>
<td>14mm</td>
<td>16mm</td>
</tr>
<tr>
<td>Extract Methanol Extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aluminium Sulphate</td>
<td>12mm</td>
<td>13mm</td>
<td>10mm</td>
<td>10.5mm</td>
</tr>
<tr>
<td>Water</td>
<td>0mm</td>
<td>0mm</td>
<td>0mm</td>
<td>0mm</td>
</tr>
<tr>
<td>Methanol</td>
<td>5mm</td>
<td>9mm</td>
<td>11mm</td>
<td>8mm</td>
</tr>
</tbody>
</table>

5. A pilot water treatment plant using natural materials at government technical College, Njinikom, Bamenda, Cameroon

The Phytobiotechnology Research Foundation (PRF), Cameroon, in collaboration with the School of Chemical Engineering, The University of Adelaide, South Australia, is proposing to carry out a capacity building training on: A simple Moringa- sand based water filtration technology for clean potable water supply in the rural schools and villages in Boyo Division, Cameroon. This is part of a doctoral research in chemical engineering, The University of Adelaide, south Australia. Three undergraduate students in chemical engineering are undertaking their honours thesis on the water quality, management and training, safety and ethical issues associated with the implementation of Integrated biocoagulant-sand filter system for drinking water purification at Government technical college, www.intechopen.com
Njinikom, Cameroon. A well with an approximate water volume of 2500 litres has been dug, and a filtration system using Moringa oleifera seeds and sand filter is being constructed expected to purify 2000 litres of water in 24 hours retention time to serve more than 7000 students.

5.1 Anticipated benefits
- Clean potable water will be available for rural people.
- Decimation of incidence of infectious/waterborne diseases
- Improved health

5.2 Training method
The training shall be conducted in conjunction with local NGOs in Bamenda, Cameroon. PRF is an NGO based in Bamenda and has a track record on community development projects in Cameroon and Nigeria. PRF has entry points to communities and has over the years worked with a number of Research institutes in the country. PRF has facilitated a number of training for local groups in Bamenda water quality in rural areas. Similarly, PRF has participated at training on water filtration technology at the ZERI Centre in Nigeria. Five (5) selected people from the local schools shall be trained and then the school authority shall provide them the resources to mount the outfits. The students will be encouraged to set up a household filter unit in their homes during holidays.
These trainees shall function in union with the PRF and the school authority who will in turn monitor and supervise effective functioning of the filter units.

6. Conclusion and recommendation

The research work has shown that there are many natural materials available in many communities in the world that can be used to treat water for drinking. Additionally, this research has demonstrated that these plant and geological materials can be applied in the treatment of any type of polluted water. These materials are ecological, low cost when compared to the application of synthetic chemicals currently used in water pollution management. The ongoing pilot system applying natural materials in water treatment in Cameroon could be replicated elsewhere. More research into the use of natural materials in water pollution management should be studied.

7. Acknowledgement

The authors would like to thank the University of Adelaide, South Australia for a PhD scholarship to do this work. The Phytobiotchnology research Foundation, Cameroon and the Principal of GTC njinikon, Cameroon for provision of funds to set up the pilot work and research.

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www.lne.uk/engineering/staff/sutherland/Moringa/cultivation/cult.htm


www.intechopen.com


Bina (1991) Investigation into the use of Natural plant coagulants in the removal of bacteria and bacteriophage from turbid waters, PhD thesis, University of New Castle Upon Tyne


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Chapter 9


Pritchard, M; Mkandawire, T;Edmondson, A;O’Neill, J.G and Kululanga, G (2009) potential of using plant Extracts for purification of Shallow well water in Malawi. physics and Chemistry of the Earth, 34: 799-805


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Water pollution is a major global problem that requires ongoing evaluation and revision of water resource policy at all levels (from international down to individual aquifers and wells). It has been suggested that it is the leading worldwide cause of deaths and diseases, and that it accounts for the deaths of more than 140,000 people daily. In addition to the acute problems of water pollution in developing countries, industrialized countries continue to struggle with pollution problems as well. Water is typically referred to as polluted when it is impaired by anthropogenic contaminants and either does not support a human use, such as drinking water, and/or undergoes a marked shift in its ability to support its constituent biotic communities, such as fish. Natural phenomena such as volcanoes, algae blooms, storms, and earthquakes also cause major changes in water quality and the ecological status of water. Most water pollutants are eventually carried by rivers into the oceans.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

CHAPTER 10

IN VITRO SENSITIVITY OF AEROMONAS HYDROPHILA TO FIVE POLARITY BASED SOLVENT EXTRACTS OF MORINGA OLEIFERA, ALUM AND CHLORINE.

Yongabi\textsuperscript{1} K.A., Lewis D.M\textsuperscript{1}

\textsuperscript{1} School of Chemical Engineering, the University of Adelaide, South Australia

Article 7 is a manuscript presented in this chapter. The results of the antibacterial activity of moringa extracts on Aeromonas is presented. This is the first report on the polarity based solvent extracts of MO on aeromonas. The activity shows that MO is better in disinfecting aeromonas from water than with alum and chlorine. The manuscript has been submitted to Chemical Engineering Science Journal, a high impact journal and ranked A\textsuperscript{3} by Excellence in Research for Australia.

The article is included in the pages that follow.
In vitro Sensitivity of Aeromonas hydrophila to five Polarity-based Solvent Extracts of Moringa oleifera, Alum and Chlorine

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School of Chemical Engineering, The University of Adelaide, South Australia
1. School of Agriculture and Wine Science, The University of Adelaide, South Australia
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Abstract
This study was undertaken to determine the in vitro antibacterial activity of five polarity based solvent extracts of Moringa seeds, seeds and shell mixture on Aeromonas hydrophila isolate from a water source. The overall purpose of this study was tailored toward addressing the problem of increasing resistance of Aeromonas hydrophila to chlorine and current antibiotics. Linear comparison of antibacterial activity with Aluminium sulphate and Chlorine was done. Aqueous extract of Moringa seeds excluding its shells showed marked antibacterial activity(29mm) on Aeromonas hydrophila greater than with sodium hypochlorite and Aluminium sulphate. The inhibitory zones produced by aqueous extract of Moringa oleifera seed and shell mixture were slightly lower with sodium hypochlorite but greater than with Alum. All the other solvent extracts except hexane had no in vitro antibacterial activity. The minimum inhibitory concentration(MIC) range (between 0.0125 mg/ml to 0.625mg/ml)for the aqueous extract of Moringa oleifera seeds extracts were lower than with sodium hypochlorite(between 0.05 to 0.0125mg/ml to 0.0125mg/ml) and Alum(0.02 to 0.05mg/ml) suggesting that at very low concentration, aqueous extract of MO could be a better disinfectant of Aeromonas hydrophila than with chlorine and alum. Microscopic observations of Moringa extracts suggest that the active ingredient is of a very small particle size less than one micron and exist in clumps. The PH of the aqueous extract of MO and Alum was acidic (3.3 and 4.5 respectively) as opposed to 7.2 for sodium hypochlorite. Findings from this study has demonstrated that aqueous extracts from Moringa oleifera seeds have a reasonable antibacterial effect on Aeromonas hydrophila isolate and can be applied in domestic water and wastewater disinfection as well apotential alternative antibacterial agents for many diseases caused by Aeromonas.

Keywords:aeromonas,water, antibacterial, moringa, extracts, alum, chlorine
Introduction

Aeromonas hydrophila is an emerging food and water borne pathogen of increasing public health importance (Bottarelli and Ossiprendi 1999). Aeromonas species are recognized as etiological agents of a wide range of diseases affecting man and animals (Rafael et al. 200; Khalifa et al. 2008). Some species have been implicated in diseases of humans such as wound infections (cellulitis), colitis respiratory infections, septicaemia, meningitis, peritonitis and occasionally urinary tract infections (Michael 1991; Rogo et al. 2009). Several food and water borne outbreaks as well as nosocomial outbreaks linked to aeromonads have been reported (Khalifa et al. 2008; Sima and Fuad 2004) particularly in developing countries (Rogo et al. 2009).

Concentration of aeromonas in ground water is generally less than 1 CFU/ml (Rogo et al. 2009) but drinking water immediately leaving the treatment plant may contain 0-10^2 cfu/ml, with potentially higher concentration in drinking water distribution systems (USEPA 2005). This has been attributed to biofilm formation (Payment et al. 1988). Higher densities of aeromonas up to 10^5 cfu/ml have been observed in waste waters, treated and crude sewage (Holmes et al. 1996).

Aeromonads have not only been isolated from sinks, drain pipes and household effluents (Arujo et al. 1991), but also from a variety of foods including, meat from lambs, porks, beef, poultry, fish (USEPA 2005). Aeromonas hydrophila are gram negative, non spore forming rod-shaped, facultative anaerobe, motile by polar flagella and grows within a wide range of temperatures (0-40°C) (Cheesbrough, 2005). Taxonomically, four major species of aeromonas were known; A hydrophila, A caviae, A sobria (presently called A veroni biovar sobria and A salmonicida which was previously classified with vibrioaceae and shigelloides (Sawetz 2004). However, the recent edition of bacteria systematic with the application of DNA technology, has classified aeromonas as a separate family: aeromonadaceae. This organism is receiving increased attention due to its resistance to a range of antibiotics and antiseptics. High incidence of antibiotic resistance of Aeromonas hydrophila, including third generation antibiotics such as cephalosporins and fluoroquinolones has been observed (Khalifa et al. 2007; Rogo et al. 2009). For instance, antimicrobial resistant strains of aeromonas isolated from water points in Istanbul, Turkey indicated that 55% A. hydrophila strains were resistant to ampicillin while 48% of the strains were resistant to erythromycin. This report as well as many have concluded that Aeromonas is a health potential risk (Koksal et al. 2007). Fluoroquinolones have been reported as the first choice treatment for Aeromonas infections, microorganisms resistant to
nalidixic acid and susceptible to ciprofloxacin are known to already have a mutation in the gyrA gene. For this purpose, the prevalence and distribution of aeromonas in clinical specimens, aquatic environments, its role as a contaminant of drinking water supplies especially in chlorinated water systems requires more attention (Siwa et al. 2004; Khalifa et al. 2007; Rogo et al. 2009)). This bacterium was frequently reported in chlorinated water (Massa et al. 1999; Burke et al. 1984b; Hazen et al. 1978; Knochel 1996; Maillard et al. 1998; Ridgeway et al. 1982 This study was focussed on the application of extracts Moringa oleifera as a possible disinfectant alternative for Aeromonas.Moringa oleifera Lam is a pantropical plant that has been used as a coagulant and antimicrobial to treat water for several years (Jahn, 1986; Sutherland et al. 1994; Muyibi 2003; Yongabi 2004; Kebreab et al, 2005; Pritchard et al2009 and Amir et al 2010). Extracts of the plant contain antibacterial activity against a wide range of bacteria and fungi (Kebreab et al, 2005; Raheela et al. 2009) but no report exist on the antibacterial activity of extracts on Aeromonas hydrophila from water source in comparison with aluminium sulphate and sodium hypochlorite used in water treatment. The study details a report on the antibacterial activity of polarity based solvent extracts from Moringa oleifera seeds,alum and chlorine on an environmental strain of Aeromonas hydrophila.

Collection of Aeromonas hydrophila wild strain
The bacterial strain was collected from Institute of Medical and Verterinary Sciences Research Institute (IMVS) at the Royal Adelaide hospital, South Australia. The bacterial was previously isolated from a water source, stored on agar slants, identified and characterized using standard protocols.

Confirmation of Aeromonas hydrophila
Aeromonas hydrophila strain was further confirmed by the method of Nzeako et al. (2002). A loopful of the culture on slant was inoculated onto pre enriched alkaline peptone water (Oxoid,PH 9) and incubated at 37°C for 24 hours. Subsequently, it was subcultured onto MacConkey agar plates (Oxoid which was previously prepared according to manufacturer’s instruction) as well as on sheep-blood agar (5% sheep blood) supplemented with 10mg/l ampicillin (SBAA). This was followed by incubation at 37°C for 24 hours. Ampicillin-resistant β-haemolytic colonies that appeared greyish white, stippled and translucent on SBAA with inability to ferment lactose on MacConkey agar was gram stained. Microscopic examination of colonies revealed gram negative rods which was
further supportive of Aeromonas identity. A subculture on nutrient agar (oxoid) slants was carried out as presumptive A. hydrophila.

Biochemical Characterization of the isolate

Ampicillin-resistant β-hemolytic colonies on SBAA and non lactose fermenting colonies on MacConkey agar were subjected to indole, methyl red, voges-proskauer, citrate (IMVIC test) with inoculation on Kliger iron Agar (KIA) slants (oxoid). The colonies were positive for IMVIC reaction, glucose and gas production, lactose negative reactions as well as oxidase positive reaction. The colonies were examined for motility in distilled water (Cheesbrough, 2005) and confirmed according to the method of Cowan (1993). The isolate was maintained on nutrient agar slants for susceptibility tests with Moringa extracts, alum and chlorine.

Sources, Identification and Processing of Moringa Oleifera Seeds

Moringa oleifera seeds were selected based on its use as a coagulant in water treatment (Jahn, 1986; Kebreab et al. 2005 and Pritchard et al, 2009). The plant has been used in many parts of Africa and India to purify water for several years. The seeds used in this study were collected from Bamenda, in the North West province of Cameroon. The plant was authenticated by botanist in the ATBU department of biological sciences, Bauchi, Nigeria. The seeds were harvested, deshelled and dried under shade at 25°C for two weeks. The seeds were then pulverized using a kitchen blender and sieved using a sieve mesh of 0.3 mm. Approximately 500 grams of the powder was stored in brown Khaki envelopes ready for extraction.

Extraction Procedures

The polarity based sequential extraction protocol was applied (Cannel, 1998). This approach of extraction has not been used in the extraction of Moringa oleifera constituents. The techniques employ a range of organic solvents from highly non polar solvent to highly polar solvent on the same plant material (Balandrin et al., 1985). One hundred gram (100g) of the dried and pulverized Moringa seeds was weighed using a digital weighing balance and steeped into 500mL of n-hexane in a 500mL conical flask. The ratio of solvent to plant material was 1:5 Weight/Volume. This was allowed to extract for 72 hours (Yongabi et al. 2009a, b). The extract was filtered using Whatman filter paper No. 13 (Whatman, UK) and the solvent in the filtrate was evaporated under a gentle stream of liquid nitrogen. The plant
residue was dried and steeped in 500mL of toluene. The same procedure was repeated for each solvent in the order; n-hexane→ toluene → acetone → methanol → water.

The resulting extracts were stored in screw capped bottles and kept at room temperature for bioassay test against Aeromonas hydrophila strain.

Determination of Antibacterial Activity of Moringa Oleifera Extracts, Aluminum Sulphate and Sodium Hypochlorite on Aeromonas Hydrophila

The agar diffusion method of Collins et al. (1995) was used. Zero point two grams (0.2g) of each of the extracts was reconstituted in 5mL of distilled water as well as 0.2g of aluminium sulphate and sodium hypochlorite was reconstituted in 5mLs of distilled water, reverse osmosis treated water free from chlorine was used.

Wells of 6mm in diameter were created in nutrient agar plates using a brass metal borer. Three hours peptone water culture of Aeromonas hydrophila was inoculated into the plates and 0.2mL of each of the extracts, of Moringa oleifera, was added to each well. A control was set up by introducing 0.2mL each of extracting solvents (distilled water, hexane, toluene, acetone and methanol) into the different wells; a free well was also left with no treatment. Wells containing 0.2mLs sodium hypochlorite, aluminium sulphate were made. The set-up was done in triplicates and plates incubated at 37°C for 24 hours. The development of zones of inhibition around the wells containing the extract indicated the antibacterial activity of the plant extract, aluminium sulphate and sodium hypochlorite against Aeromonas hydrophila. The differences between the zones observed for the test and that of the control was recorded as the actual diameter of zones of inhibition caused by the Moringa oleifera extracts, sodium hypochlorite and aluminium sulphate.

Determination of Minimum Inhibitory Concentration (MIC) of Moringa Oleifera Extracts, Aluminium Sulphate and Sodium Hypochlorite

Zero point five millilitre (0.5mL) of the reconstituted extracts (200mg/mL) of Moringa oleifera active aqueous extract, aluminium sulphate and sodium hypochlorite was placed in test tubes (No. 1 - 8) containing peptone water.

The first tube contained 1.5mL of peptone water while tubes 2 to 8 contained 1.0mL of peptone water each. Tube 8 served as the control experiment. After serial dilution, the final concentration of each tube was 50mg/mL, 12.5mg/mL, 3.12mg/mL, 0.25mg/mL, 0.20mg/mL, 0.02mg/mL and 0.0125mg/mL respectively. The tubes were inoculated with a loopful of the test organism (Aeromonas hydrophila) and incubated at 37°C for 24 hours.
At the end of the incubation, the lowest dilution which showed no visible turbidity or inhibitory zone was regarded as the minimum inhibitory concentration (Prescott et al. 1990). The same procedure was used to determine the MIC for aluminium sulphate (Alum) and sodium hypochlorite.

Results
The nature and yield of extracts from seeds and shells of Moringa oleifera shows that the water extract had a higher yield (Table 1). The hexane, toluene and acetone extracts from the seeds of Moringa oleifera were oils (Table 1, 2).

Hexane extracts had a higher oil yield (Table 1, 2). The oil yield from the seeds without shells higher than from the seeds with shells (Table 1, 2).

The results (Fig 1) showed that the hexane extract of the Moringa seeds and shells mixture exhibited antibacterial activity against Aeromonas hydrophila. The water extract had the highest zone of inhibition, closely followed by sodium hypochlorite and then aluminium sulphate.

All the solvent controls showed no inhibition. The results of the antibacterial activity of Moringa seeds without shell extracts showed that the hexane extracts had no inhibition (Fig.2). The aqueous extract had an activity lower than the activity of the seeds and shells mixture. The zones of inhibition of Mo seeds without shell are lower than with sodium hypochlorite but higher than with aluminium sulphate (Fig.2).

The minimum inhibitory concentration (MIC) for Aeromonas hydrophila ranged from 0.0125mg/mL to 0.625mg/mL for aqueous extract of Mo (Table 6), 0.05mg/mL to 0.0125mg/mL for sodium hypochlorite and 0.023mg/mL to 0.05mg/mL for aluminium hypochlorite. The aqueous extract of Mo had activity at very low concentration when compared to sodium hypochlorite and aluminium sulphate (Table 4,5). The pH of the aqueous extract of Mo was 4.18 (Table 3) while pH of aluminium sulphate was 3.55 sodium hypochlorite had an alkaline pH (Table 5). Fig 3a shows the microscopic shot of Moringa oleifera aqueous extract. This suggest that the active ingredient is less than one micron and exist in clumps or bound to each other. Fig 3b shows the seeds of Moringa encased in its shells. Fig 4 shows marked inhibition zones exhibited by the aqueous extract of MO seeds on Aeromonas hydrophila on nutrient agar medium. Fig % a shows the nature of Aeromonas hydrophila cells before treatment with aqueous extract of MO, while Fig 5b shows lysed Aeromonas hydrophila cells attached onto Moringa active ingredient. The debris suggest that the bactericidal activity of the extract on Aeromonas cells. A Preliminary thin layer chromatographic analysis of the active aqueous Mo extract showed no separation in a
Chapter 10

combination water and Methanol in a ratio of 50%:50% and 50% Hexane and 50% methanol (polar and non polar solvent system).

Table 1: Nature and Yield of Extracts from seeds of Moringa oleifera and Shell mixture per 50 grams sample.

<table>
<thead>
<tr>
<th>Extract Fraction</th>
<th>Nature of Extract</th>
<th>Extract Yield</th>
<th>Percentage Yield(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>Oil, greenish yellow</td>
<td>10.97</td>
<td>21.95</td>
</tr>
<tr>
<td>Toluene</td>
<td>Oil yellow</td>
<td>2.74</td>
<td>5.48</td>
</tr>
<tr>
<td>Acetone</td>
<td>Crystal, oily pellets</td>
<td>7.41</td>
<td>14.80</td>
</tr>
<tr>
<td>Methanol</td>
<td>Brown liquid, slightly oily</td>
<td>5.050</td>
<td>10.20</td>
</tr>
<tr>
<td>Water</td>
<td>Creamy liquid</td>
<td>23.6</td>
<td>47.2</td>
</tr>
</tbody>
</table>

Table 2: Nature and Yield of Extracts from Seeds of Moringa oleifera Seeds Without Shells) per 50 gram sample.

<table>
<thead>
<tr>
<th>Extract Fraction</th>
<th>Nature of Extract</th>
<th>Extract Yield</th>
<th>Percentage Yield(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td></td>
<td>8.81</td>
<td>17.6</td>
</tr>
<tr>
<td>Toluene</td>
<td></td>
<td>8.75</td>
<td>17.5</td>
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<tr>
<td>Acetone</td>
<td></td>
<td>12.78</td>
<td>25.6</td>
</tr>
<tr>
<td>Methanol</td>
<td></td>
<td>5.2</td>
<td>10.4</td>
</tr>
<tr>
<td>Water</td>
<td></td>
<td>23.6</td>
<td>47.2</td>
</tr>
</tbody>
</table>
Chapter 10

Fig. 1. Comparative antibacterial activity of solvent extracts of Moringa oleifera seeds and shell mixture, Aluminium sulphate and Sodium hypochlorite on Aeromonas hydrophila.

Fig 2. : Comparative antibacterial activity of solvent extract Moringa oleifera seeds without shells, Aluminium sulphate and sodium hypochlorite on Aeromonas hydrophila.

Table 3: PH of Aqueous Extract of Mo, Aluminium Sulphate and Sodium hypochlorite

<table>
<thead>
<tr>
<th>Parameter</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water Extract of Mo</td>
<td>4.5</td>
</tr>
<tr>
<td>Aluminium Sulphate</td>
<td>3.3</td>
</tr>
<tr>
<td>Sodium Hypochlorite</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Table 4: Minimum Inhibitory Concentration (MIC) Value of Aqueous Extract of Mo on Aeromonas hydrophila
Table 5: MIC Values for Sodium Hypochlorite and Aluminium Sulphate on Aeromonas hydrophila

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Hypochlorite</td>
<td>Aluminium Sulphate</td>
</tr>
<tr>
<td>0.04</td>
<td>22.5, 22.0</td>
</tr>
<tr>
<td>0.02</td>
<td>10.5, 10.5</td>
</tr>
<tr>
<td>0.005</td>
<td>4.7</td>
</tr>
<tr>
<td>0.0125</td>
<td>Not _____</td>
</tr>
<tr>
<td>0.0625</td>
<td>Not _____</td>
</tr>
</tbody>
</table>

*MIC range for sodium hypochlorite between 0.05 to 0.0125mg/ml

*MIC range for aluminium sulphate between 0.02 to 0.05mg/mL

Fig 3a. Microscopic appearance of Aqueous shells
active extract showing coagulant, antibacterial protein in clumps.

Fig 3b. Moringa seeds enclosed in
Fig 4. Marked Zones of Inhibition of Aqueous extract of M.Oleifera seeds on Aeromonas hydrophila

Discussion

The marked inhibitory effect of aqueous extract of Moringa oleifera seeds on Aeromonas hydrophila shows the potential antibacterial active compounds in the seeds. Previous reports show that Moringa seeds possess peptides (Kebreab et al. 2005), lectins and proteins (Amir et al., 2010; Pritchard et al., 2009; Muyibi et al. 2003). Antimicrobial activity of the seed extracts of Moringa seeds extracted independently with water, hexane, ethanol, chloroform has been reported (Raheela et al. 2009; Kebreab et al. 2005; Okuda et al. 2001) against a wide range of bacteria (Pseudomonas aeruginosa, staphylococcus aureus and E coli ) and fungi (Candida albicans and Trichophyton spp). Greater antimicrobial activity has been reported in the leaves of the Moringa oleifera than with the seeds.

However, the antibacterial activity of the aqueous extract of Moringa seeds against Aeromonas hydrophila isolate has not been reported before. In this study, the activity of aqueous extract of MO seeds in comparison to sodium hypochlorite and aluminium sulphate used in traditional water treatment was done. This is a first report to demonstrate not only the antibacterial activity of Moringa seed extracts using polarity based extraction approach as well comparison with traditional coagulants and disinfectants used in present day water
Chapter 10

In order to identify a better solvent extraction system for Moringa oleifera disinfectant/active ingredient, a sequence of different polarity based solvents was used for extraction. This extraction protocol on Moringa oleifera seeds suggests a number of advantages: a higher yield of Moringa seed oil of more than 40% which has not been reported before, higher crude yield of the water extract (47.2%) not reported before. In previous studies an oil yield of up to 35% has been reported by Muyibi et al. (2003) and 33.5% (Mani et al. 2007).

The hexane extract of Moringa oleifera seeds extracted with its shells inhibited Aeromonas hydrophila. This activity was not noticed in the hexane extract of the seeds without shell. The shells may contain an antibacterial substance which is soluble only in non polar hexane. The industrial and pharmaceutical applications of the oils extracted from moringa seeds and shells could be considered. Aeromonas hydrophila causes skin infections in both man and animals and as such this oil may have a potential to treat skin infections due to aeromonas. The MIC of the aqueous extract of moringa oleifera range from 0.0125mg/mL to 0.625mg/mL while that of sodium hypochlorite ranged from 0.05mg/mL to 0.0125mg/mL and aluminium sulphate 0.02mg/mL to 0.05mg/mL. This means that higher concentrations of sodium hypochlorite and aluminium sulphate is required to disinfect Aeromonas hydrophila in water distribution systems than with the application of aqueous extract of moringa oleifera seeds. These results also suggest that, despite the occurrence of Aeromonas in chlorinated water supplies, it is still fairly susceptible to free chlorine. This observation corroborates previous observation (burke et al., 1984b). The frequent isolation of aeromonads from chlorinated water supplies could be linked to poor residual chlorine level in water distribution system. Aqueous extract of Moringa seeds could provide a solution to this problem, as very low concentrations of MO aqueous extracts are required to disinfect aeromonas hydrophila or to treat aeromonas infections.

The considerably low MIC values for MO aqueous extract may be due to alkaloids and peptides present in the seeds (Kebreab et al. 2005). This further demonstrates the advantages of using Moringa seed extracts in water treatment as well to achieve effective treatment of infectious water borne diseases. The observation in this study is in corroboration with previous studies which opined that the active ingredient in Moringa seeds is water soluble (Sutherland et al. 1994; Jahn 1986; Kebreab et al. 2005; Pritchard et al. 2009).

The findings in this study demonstrate tremendous potentials in the disinfection of aeromonas hydrophila, considering its resistant to chlorination and a wide range of antibiotics, the findings in this study may contribute in providing solutions to this problem.
The pH of the Moringa aqueous extract was acidic as well as aluminium sulphate. This may have also contributed to the antibacterial activity observed (Yongabi 2004). Moringa seed aqueous extract has demonstrated a high antibacterial activity against aeromonas hydrophila. This has a potential application in water disinfection and as an alternative antibacterial substance against a wide range of diseases caused by aeromonas. Furthermore, the results also demonstrates that the polarity based sequential extraction protocol could be the best in terms of oil yield from Moringa seeds and the quantity of the active aqueous extract.

The findings also provide insights on the possible concentrations of aluminium sulphate and sodium hypochlorite needed to effectively disinfect water.

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Chapter 10


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Chapter 10


155


CHAPTER 11

PHYTOCHEMICAL CONSTITUENTS AND COMPARATIVE ANTIFUNGAL ACTIVITY OF POLARITY BASED SOLVENT EXTRACTS OF *MORINGA OLEIFERA* SEEDS, ALUM AND CHLORINE ON *ASPERRILLUS FUMIGATUS* ISOLATE FROM WASTEWATER.

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Article 8 is a manuscript presented in this chapter. The results of the antifungal activity of *Moringa* extracts on *Aspergillus fumigatus* is presented. This is the first report on the polarity based solvent extracts of MO on waterborne fungus. The activity shows that MO is better in disinfecting *Aspergillus fumigatus* from water than Alum and Chlorine. The manuscript has been submitted to Chemical Engineering Science Journal, a high impact journal and ranked A^3 by Excellence in Research for Australia.

The article is included in the pages that follow.
Chapter 11

Phytochemical constituents and comparative antifungal activity of polarity based solvent extracts of Moringa oleifera seeds, alum and chlorine on Aspergillus fumigatus isolate from Wastewater.

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Abstract:

*Moringa oleifera* seeds were extracted using solvents ranging from non polar hexane to highly polar water and tested for their phytochemicals constituents and in vitro antifungal activity on a strain of *Aspergillus fumigatus* isolate from wastewater. In this study, the water extracts showed to have the highest percentage yield of 42.7 %. The presence of only alkaloid in the aqueous extract suggested that the highly polar solvents are better extracting solvent when compared to non polar solvents for active antifungal fraction of *Moringa oleifera* seeds. In previous studies, a peptide of approximately 12kDa identified as an alkaloid has been isolated from aqueous extracts of Moringa seeds with antimicrobial activity with no report on the antifungal activity of aqueous extract of MO on waterborne fungal isolates. Flavonoids were detected in all the extracts while steroids were found in only the polar extracts. All the extracts of *Moringa oleifera* inhibited mycelia growth of *Aspergillus fumigatus* as compared to a mycelia spread of 85mm on the control plates. The aqueous extract of MO inhibited spore germination, while no inhibitory effect was observed for Aspergillus plates treated with alum and chlorine. The presence of bioactive alkaloid in the aqueous extract of MO may suggest that the reason for the common application of Moringa seeds extracts in rural Africa in the treatment of fungal infections. This report is the first authentication of the antifungal activity of the crude polarity based extracts of *Moringa oleifera* seeds in comparison to alum and chlorine on an isolate of fungi, *Aspergillus fumigatus*, from wastewater, and suggests that Moringa seed extracts could be applied to disinfect water borne fungi while alum and chlorine has no toxic effect on water borne fungi.

Keywords: *Moringa oleifera*, Antifungal, Aspergillus, Water, Phytochemical, Chlorine.
Chapter 11

1. Introduction

Approximately five million lives are lost annually due to drinking and use of contaminated water (Madore et al., 1987; WHO, 2006 and Pritchard et al., 2009; Amir et al., 2010). Many drinking water utilities rely solely on monitoring indicator organisms such as coliforms and *E. coli* to ensure quality of water (Cheesbrough, 2005). However, the presence or absence of these indicator organisms may not correlate well with the presence of fungal contaminant in water (Dogget, 2003; Hageskal et al, 2006 and Pereira et al, 2009). Limited attention has been given to the presence of fungi in public water supply systems (Dogget, 2000; Arvanitidou et al, 2002; Hageskal et al, 2007 and Pereira et al, 2009). There is a need not only to monitor the fungal spectrum of aquatic systems but to apply sustainable techniques to decontaminate fungi from public water systems. One of technologies is to apply plant materials such as *Moringa oleifera*. *Moringa oleifera* consist of a tremendous variety of secondary metabolites with diverse applications in water treatment and in clinical medicine (Muyibi and Evison, 1995b; Kebreab et al, 2005 and Pritchard et al, 2009). The interest in studying *Moringa oleifera* has increased since Jahn in 1979 reported its significant role as a coagulant in water purification and antimicrobial activity (Olsen, 1987 and Raheela et al., 2009). Many bioactive compounds in *Moringa oleifera* plants have been found to have anticancer, antibacterial, antifungal and immunostimulatory properties (Fuglie, 2001). Despite the uses of Moringa extracts for water treatment and pharmaceutical applications, researchers have focussed their interest solely in the isolation of coagulant active ingredient (Okuda et al., 1999 and Okuda et al., 2001b) and antibacterial studies with limited studies on the application of Moringa seeds to disinfect waterborne fungi.

*Moringa oleifera* is widely used in folk medicine, as a human health supplement and also as animal feed due to its high protein content and high concentration of essential amino acids, vitamins, minerals and fatty acids (Fuglie, 2001). The detection of primary and secondary metabolites in the leaves, roots and seeds has been employed in various pharmaceutical applications. For example, the primary and secondary metabolites of *Moringa* have the potential as an antiviral, antibacterial, anti-inflammatory, anti-tumour as well as anticancer activity (Amanda et al., 1991; Fuglie, 2001). Coagulant and antimicrobial peptides of less than 10 kDa using various extraction and purification protocols has been reported (Ndabigengesere et al., 1995; Kebreab et al, 2005; Dorries, 2005) and an antimicrobial alkaloid called pterygospermin was reported by Eilert in 1981.

Independent solvent extracts of *Moringa oleifera* seeds were screened and reported that the water, acetone, petroleum ether and diethyl ether extracts has considerable
antimicrobial activity against many microorganisms (Dorries, 2005; Kebreab et al., 2005; Raheela et al., 2008 and Amir et al., 2010). Nevertheless, it is not well understood if the phytochemical constituents responsible for the coagulant activities are the same for the disinfection activity. The importance of analysing the phytochemical constituents is due to the fact that these chemicals are the key to the biological activities. Although many studies showed the extensive use of Moringa oleifera is essential for the development of pharmaceuticals, its phytochemical constituents require continuous elucidation. Water borne fungi such as Aspergillus spp has been implicated in many immunocompromised diseases such as cancer, HIV and AIDS (Cheebrough, 2005). Therefore, in this study, the preliminary phytochemical screening of Moringa oleifera and the in vitro inhibitory effect of polarity based extracts from this plant on Aspergillus fumigatus isolate from a wastewater source were investigated in comparison to Alum and Chlorine.

2. Material and methods
2.1 Collection, Processing and Extraction procedure of Moringa oleifera seeds.
Moringa oleifera seeds were obtained from Bamenda in Cameroon where it is used in many rural areas to treat water. The seeds were dehusked and pulverized using a kitchen blender then sieved using a 0.01 sieve mesh. Fifty grams of the powder was added to 250ml of five solvents sequential extraction (1:5, w/v) in 250ml beakers (Pyrex). The extraction was first carried out for a period of 48 hours with hexane and the extracts were filtered by gravity filtration. The solvents were evaporated under a gentle stream of nitrogen gas with a warm water bath (at 50°C). The extraction was repeated with the residue by adding in toluene, acetone, methanol and finally distilled water (1:5, w/v) in the same manner, except the aqueous extract was dried under sunlight. The weight of the extracts was obtained to calculate the percentage yield (Cannel, 1998).

2.2 Analyses for phytochemical constituents
All of the sequential extracts were subjected to preliminary phytochemical screening as described by Geissman (1963), Trease & Evans (1989) and Harborne (1998). The preliminary screenings were carried out to identify alkaloids, tannins, saponins, phlobatannins, cardiac glycosides, steroids, terpenoids and flavonoids. For tannins, FeCl₃ test was used; for alkaloids, Wagner test was used; for cardiac glycosides, Keller killiani test was used; for saponins, frothing test was used; for phlobatannins, hydrochloric acid was used for testing; for flavonoids, alkaline solution and diluted acid were used for testing; for
2.3. Isolation protocol and identification of Aspergillus fumigatus

Wastewater sample from a mining well was cultured on Potato dextrose agar using the pour plate technique (Collins et al, 1995). One millilitre of aliquots of the wastewater were aseptically incorporated on to molten agar and swirled gently to have a homogenous mixture. This was done in triplicates and plates incubated at room temperature (23°C) and growth monitored daily for one week. Mycelia strands and spores were picked using an inoculating loop and a smear made using lactophenol cotton blue stain. This preparation was examined microscopically and characteristic micro morphology of Aspergillus such as ascospores and greenish mycelia strands were indicative of *Aspergillus fumigatus*. Specific taxonomic guides specified by David et al (2007) were used to confirm the identity of this organism.

2.4 In Vitro Antifungal assay

Antifungal screening of the sequential extracts was conducted using the agar diffusion method (Collins, Lyne, & Grange, 1995). Tested organism (*Aspergillus fumigatus*) was subcultured and grown on potato dextrose agar at 27°C for 7 days. The sequential extracts were dissolved in its extracting solvent and made up to a concentration of 40 mg/ml except the aqueous extract which was already in liquid form. Each of the extracts, their corresponding extracting solvents as controls, Alum and Chlorine were carefully incorporated into molten potato dextrose agar plates in triplicate, swirled gently to have a homogenous mixture. They were then allowed to set. After inoculation, a 6mm diameter of the test fungus (mycelia block) with approximately 5000 spores per ml was aseptically placed at the centre of each plate. The set up was incubated at room temperature and growth rates monitored daily by direct measurement for up to two weeks. The extracting solvents, such as hexane, toluene, acetone, methanol and water; were included as a positive controls. The actual growth inhibition of the test organism was examined by measuring the rate of spread of the mycelium and spore germination ability.

3. Results and Discussion

The appearance and the yield of the sequential extracts of Moringa seeds were observed and presented as in Table 1. The yield of the sequential extracts ranged from 5.4% to 47.2% with the aqueous extract having the highest percentage yield.
The potential antimicrobial active compounds are mostly found from the organic solvent extracts (Kebreab et al., 2005). Coagulant and antimicrobial peptides less than 10kDA have been detected from bulk water extracts of Moringa oleifera seeds (Kebreab et al., 2005 and Amir et al., 2010). This, however, has not been tested on any fungal isolate from water or wastewater. Previous studies also showed that the organic solvent extracts from *Moringa oleifera* have been found to show antimicrobial activity (Raheela et al., 2008; Mala et al, 2009; Amir et al., 2010). In order to identify a better solvent extraction for *Moringa oleifera* seeds, a sequence of different polarity solvents were used for the extraction. In this study, all the extracts displayed varying antifungal activity against *A. fumigatus* (Table 5). The extracting solvents, Alum and Chlorine had no antifungal activity against *Aspergillus fumigatus* (Figs 1 and 2). The finding in this study is in agreement with the previous report of antifungal activity of Moringa seeds (Raheela et al., 2008). However, the fungi used in previous reports were obtained from skin infection. Extracts from Moringa have not been tested on water borne fungi, particularly *Aspergillus fumigatus*.

Preliminary screening of different sequential extracts for alkaloids, tannins, saponins, phlobatannins, cardiac glycosides, steroids, terpenoids and flavonoids was evaluated in Tables, 1 and 3.

The phytochemical screening of the aqueous extracts of *Moringa oleifera* revealed the presence of alkaloids. Greater antifungal activity was observed with the aqueous extract than with all the other solvent extracts. The aqueous extract totally inhibited spore germination. This observation has not been reported elsewhere. This finding possibly lends credence to the reason why most researchers prefer to use water and methanol extracts of medicinal plants for the antimicrobial testing. From these results, the polarity based sequential extraction protocol may be a better extraction solvent system than the use of one single solvent for extracting active components from *Moringa oleifera* seeds.

Flavonoids were discovered in all the extracts of *Moringa oleifera* seeds. This suggests that they are soluble in both polar and non polar solvents. This phyto-constituents have been reported to have, anti-inflammatory, anti-diabetic, anti-viral, anti-fungal and antibacterial properties; moreover, it plays a significant role in the central nervous system activities (Argal & Pathak, 2006; Lacaille-Dubois & Wagner, 1996; Milgate & Roberts, 1995; Rupasinghe, et al., 2003; Sayyah, Hadidi, & Kamalinejad, 2004). The industrial applications of oils from *Moringa* have also increased significantly due to its important role as a drug for skin infections. Steroids were detected only in the acetone, methanol and water extracts. This implies that steroids are highly soluble in polar solvents. Steroids are found to have the similar role as saponins, both of these bioactive compounds are
Chapter 11

responsible for central nervous system activities (Argal & Pathak, 2006). In addition, steroids showed to possess analgesic and anti-inflammatory effect (Lerner, Bianchi, Turkheimer, Singer, & Borman, 1964; Sayyah, et al., 2004). The anabolic-steroids, of which are the most common drugs in this class of phytochemicals, has been used by more than 1 million people in the United States (DuRant, Escobedo, & Heath, 1995). These results seem to support this finding since polar extracts from Moringa seeds possess steroid and might have the potential to show antifungal activity. Flavonoids were found in all the extracts of Moringa seeds. There are many reports suggesting that flavonoids have anti-tumour, and anti-inflammatory effect (Al-Meshal, Tariq, Parmar, & Ageel, 1986; Wang, Xia, Yang, Natschke, & Lee, 1998). Flavonoids isolated from citrus fruits also demonstrated anticancer properties both in vivo and in vitro activity (Silalahi, 2002).

4. Conclusion

In conclusion, the presence of these phyto-constituents suggested that extracts from Moringa oleifera seeds are a good antifungal agent for disinfection of Aspergillus spp from contaminated water. This observation has not been reported elsewhere. In addition, the solvents used in the extraction play an important role to obtain the phyto-constituents from Moringa oleifera seeds. Aluminium sulphate and sodium hypochlorite did not exhibit any antifungal activity against this isolate. This suggests that Alum and Chlorine may not have any effect in disinfecting water borne fungi. This is a very important observation that points to the fact that fungi monitoring in water analysis should be considered, as traditional faecal indicators may not correlate well with this group of organisms. Furthermore, Moringa oleifera seed extracts could be applied in disinfecting water borne fungi. The findings also suggest that Moringa seed extracts could also be applied to treat clinical infections such as systemic mycosis and Aspergillosis in immunosuppressed patients.
## Chapter 11

### Table 1: Phytochemical Components/Constituents of Moringa seeds and Shells

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Hexane Extract</th>
<th>Toluene Extract</th>
<th>Acetone Extract</th>
<th>Methanol Extract</th>
<th>Water Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
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</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**Key**
- → not detected  
+ → detected

### Table 2: Phytochemical Components/Constituents of Moringa seeds without Shells

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Hexane Extract</th>
<th>Toluene Extract</th>
<th>Acetone Extract</th>
<th>Methanol Extract</th>
<th>Water Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**Key**
- → not detected  
+ → detected
### Table 3: Nature and Yield of Extracts from seeds of Moringa oleifera (Lam) per 50 grams Samples + Shells

<table>
<thead>
<tr>
<th>Extract Fraction</th>
<th>Nature of Extract</th>
<th>Extract Yield</th>
<th>Percentage Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>Oil, greenish yellow Butterlike, viscous</td>
<td>10.97</td>
<td>21.95</td>
</tr>
<tr>
<td>Toluene</td>
<td>Oil (viscous) yellow Less viscous</td>
<td>2.74</td>
<td>5.48</td>
</tr>
<tr>
<td>Acetone</td>
<td>Crystal like oil Pellet-like</td>
<td>7.41</td>
<td>14.82</td>
</tr>
<tr>
<td>Methanol</td>
<td>Brown liquid Slightly oil, creamy</td>
<td>5.080</td>
<td>10.20</td>
</tr>
<tr>
<td>Water</td>
<td>Creamy liquid</td>
<td>23.6</td>
<td>47.2</td>
</tr>
</tbody>
</table>

### Table 4: Nature and Yield of Extracts from Seeds of Moringa (Lam) Seeds without Shells per 50 gram Sample

<table>
<thead>
<tr>
<th>Extract Fraction</th>
<th>Nature of Extract</th>
<th>Extract Yield</th>
<th>Percentage Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>Oil, light brown Butter consistency</td>
<td>8.81</td>
<td>17.6</td>
</tr>
<tr>
<td>Toluene</td>
<td>Oil, light yellow Butter-like Consistency</td>
<td>8.71</td>
<td>17.5</td>
</tr>
<tr>
<td>Acetone</td>
<td>Pellet-like oils Butter consistency</td>
<td>12.78</td>
<td>25.6</td>
</tr>
<tr>
<td>Methanol</td>
<td>Brownish oil/greasy liquid</td>
<td>5.2</td>
<td>10.4</td>
</tr>
<tr>
<td>Water</td>
<td>Gummy, sticky Resinous, semi brownish liquid</td>
<td>23.6</td>
<td>47.2</td>
</tr>
</tbody>
</table>
Table 5: Inhibitory Effect of Moringa Extracts, Alum, and Chlorine on Aspergillus fumigatus isolate from waste water

<table>
<thead>
<tr>
<th>Extracts Control</th>
<th>6 mm Inoculums initially with approx 10,000 spores per mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1 2 3 4 5 6 7 8 9 10 12 14</td>
</tr>
<tr>
<td>Aspergillus Fumigatus Control Culture Plate</td>
<td>6 mm 15 35 42 50 58 7 2</td>
</tr>
<tr>
<td>HexaneMo</td>
<td>6 6 6 6 6 6 6</td>
</tr>
<tr>
<td>MethanolMo</td>
<td>6 6 6 6 6 6 6</td>
</tr>
<tr>
<td>Salt Mo</td>
<td>6 6 6 6 6 6 6</td>
</tr>
<tr>
<td>Crude Mo Extracts</td>
<td>6 6 6*1 6 6 6</td>
</tr>
<tr>
<td>Aqueous Sequential Mo Extract</td>
<td>6 6 6*1 6 6 6</td>
</tr>
<tr>
<td>Aluminium Sulphate</td>
<td>6 15 34*2 42 50 56 85 mm</td>
</tr>
<tr>
<td>Sodium Hypochlorite</td>
<td>6 13 34*3 42 50 56 85 mm</td>
</tr>
</tbody>
</table>

*1 → No spore germination  
*2 → Prefuse sporulation  
*3 → Sporulation

Fig.1 Aspergillus fumigatus control plate and plate showing inhibition by crude aqueous extract of Moringa oleifera seeds.
Fig 2: Confluent growth of Aspergillus fumigatus on plate treated with Chlorine.

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Chapter 11


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CHAPTER 12

AN APPROPRIATE TECHNOLOGY TRANSFER. A SUSTAINABLE LOW COST PHYTODISINFECTANT- SAND FILTER ALTERNATIVE FOR WATER PURIFICATION

Figure 25 Phyto disinfectant - sand filter.
(Yongabi et al.,2012)

Figure 26 Pollution sources, Cameroon (Yongabi,2010)

INTRODUCTION
As discussed in Article 2 (Chapter 5), there are many plants in Africa with phytocoagulant and phytodisinfectant potentials (Articles 1, 2, and 3 – Chapters 4-6). The
future of water treatment technology in Africa lies in the use of natural alternative materials for a range of reasons. The materials are widespread, and easy to use. Additionally, polyelectrolytes in water treatment applications are expensive (Article 1, Chapter 4), harmful and requires training (Articles 1, 2 and 3 – Chapters 4-6). The level of water pollution is constantly increasing (Article 6, Chapter 9). As observed by Pritchard et al. (2009) and Zang et al (2006), more than 1.2 billion people in the world do not have access to safe drinking water (Article 2 – Chapter 5). A significant contribution made in this study is the validation of the coagulant and disinfectant activities of some local plants. These have not been reported on earlier. The coagulant activity of *Moringa oleifera* observed was comparable to aluminum sulphate. This corroborate previous reports of Kebreab (2005), Yongabi (2004) and Amir et al, (2010) (see Articles 1, 2, 3 and 5 – Chapters 4-6 and 8).

Extensive reports on drinking water quality is lacking in Bamenda, Cameroon, especially in the rural areas. This work demonstrates significant contribution in reporting the level of water safety in the rural north west region of Cameroon (Articles 3 and 1 – Chapters 6 and 4). The significantly high microbial load and diversity is far above the World Health Organization and Australian water quality standards (Article 3 – Chapter 6). Extensive studies reported antimicrobial and turbidity removal properties of *Moringa oleifera* Ghebremichael et. al, (2009) and Pritchard et al, (2009) (Articles 4 and 5 – Chapters 7 and 8). In this research, similar a observation is reported with further results on bench scale work proving phytodisinfectant potentials. The latter aspects has not been previously reported (Article 3 – Chapter 6)

The antimicrobial effect of *Moringa oleifera* on *Escherichia coli* strain, *Aeromonas hydrophila* and *Aspergillus fumigatus* is reported. This significant observation especially on *Aeromonas hydrophila* has not been previously reported. *Aeromonas hydrophila* is resistant to chlorine and a range of antibiotics. (Vally et al, 2004) and Trovatelli (1999).The integration of *Moringa oleifera* into a sand filter system proved potentially efficient in treating water. In the lab scale filtration the findings generally demonstrated a disinfection rate above 99.5% and a coagulation rate of 99.9% using synthetic turbid and contaminated water. This has been observed by Pritchard et al (2009). The average flow rates were generally 2 drops per second for a 44 mm ID filter, faster than with just a slow sand filter. Bench scale filtration rigs where different organisms such as *aeromonas sp*, *candida sp* and spore forming fungi, other than indicator organisms (*E.coli*) were used in synthetic contaminated water has not been reported earlier on. Pretreated synthetic contaminated
water containing each of these organisms before filtration onto sand filter beds demonstrated more than 99.9% efficacy at an average flow rates of two drops per second.

The Moringa-sand filter integration produces clean water in a day (Articles 1,2,3,4 and 5 – Chapters 4-8)

A pilot system operating on this principle was set up and water produced in a day met the WHO standards (Articles 1 and 6 – Chapters 4 and 9). The involvement of students and the community during the construction of the pilot plant demonstrated an appropriate transfer of skills and in situ capacity building. A training manual on the effective management of the pilot water system was also developed with the use of graphics for easy follow up.
CHAPTER 13

IMPACT AND CONCLUSIONS

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Figure 27: Community mobilization with school children

Figure 28: Pilot water filter system (Yongabi et al., 2012)

This chapter concludes the Thesis by presenting an overview of the impact of this research to date, making recommendations for further and future improvements in water purification at low cost in Sub Saharan Africa. The thesis addresses a fundamental research problem, the need for a better understanding and exploration of local materials for water purification in Cameroon and its potential application in wastewater treatment. These local materials (*Moringa oleifera*, *Jatropha curcas*, *Pleurotus tuberregium* and sand) have been validated and demonstrated to have coagulant and disinfection activities similar to alum and chlorine. The outcome of this study contributes significantly to the social, economic and health status of the people in Cameroon and Africa at large. The natural coagulant inventory would provide potentially cheap and available alternatives for a sustainable water
Chapter 13

treatment in areas that cannot afford chlorine and alum. This also implies significant budgetary savings by local governments in these countries on importation of polyelectrolytes and chlorine, traditionally used for water treatment. The use of natural coagulants and disinfectants for water treatment could potentially reduce the hazards of handling chlorine and alum by the rural people. Chlorine has been reported as a precursor for carcinogenic compounds such as trichloromethane, this would ultimately mean a cut down in the risk of developing cancer. The basic water microbiology, risk of water borne infections in surface water in Cameroon is also reported for the first time. This is useful in generating awareness of the potential public health dangers and need for caution. The data generated and in published literature could be potentially be used in the future by WHO and the governments of Cameroon for strategic planning purposes. This research has contributed significantly to fundamental research in the fields of water microbiology, environmental engineering, and applied mycology. New terms such as phytocoagulants, phytodisinfectants and mycocoagulants were used for the first time in literature and cited at this link: Alternative Perspectives in Water and Wastewater Treatment

www.amazon.com/Reference-Books/. This book, covering an overview on this paradigm shift in water treatment technology, was published from this thesis research and is being used in engineering faculties of a number of universities around the world. Safe clean water is already available to more than 500 inhabitants in the study village in Cameroon with potential to disseminate this knowledge to other parts of Cameroon and Africa via planned workshops and seminars. The design of the Moringa sand filter drum is low cost and implies that each household can practically construct their own water filter unit. The improved flow rates and short retention time, unlike with a normal sand filter outfit, are significant strides demonstrated in this research. This has a great potential to treat any kind of turbid water and stands to benefit more than 70% of the populace of Cameroon and Africa at large.

A significant impact of this technology is that it can improve hygiene and sanitation for more than 70% of Africans and save energy. The impact on the environment is also worth noting. Conservation of the local plants opens up another new research frontier. The report of the antibacterial activity of Moringa oleifera extracts on aeromonas hydrophila and Aspergillus fumigatus, a waterborne fungus, is reported for the first time in this work. Chlorine and alum have no significant toxic effect on these microorganisms, thus the findings from this study could influence future research in this area. A training manual on dissemination of skills for a household filter in Cameroon has been developed, simple field tests to ascertain water quality in rural Cameroon have been developed as well as
considering safety and ethical issues on the use of this technology. It is recommended that future studies will test the efficacy of the phytodisinfectant sand filter system on heavy metal removal such as arsenic, removal of viruses and potentials in treating water with high salt concentration.
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References


References


References


References


## APPENDIX 1: SUMMARY OF DISINFECTION OF PATHOGEN CONTAMINATED WATER PRETREATED WITH MORINGA EXTRACTS BEFORE SAND FILTRATION RIG (AT 45 MINUTES RESIDENCE TIME)

THE RESULTS ARE SUBMITTED FOR FUTURE PUBLICATION.

<table>
<thead>
<tr>
<th>Pathogen/ Microbe (24 hrs broth culture)</th>
<th>MO Salt Extract sand filtration</th>
<th>MO Aqueous Sequential Ext sand filtration</th>
<th>MO Crude Ex sand filtration</th>
<th>Sand filtration Control</th>
<th>Initial Pathogen and Turbidity Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cnt Tur b</td>
<td>cnt Tur b</td>
<td>cnt Tur b</td>
<td>cnt Tur b</td>
<td>cnt Tur b</td>
</tr>
<tr>
<td>Aeromonas hydrophila (wild strain)</td>
<td>26.8 0.85</td>
<td>2.0 0.8</td>
<td>9 1.7</td>
<td>248 0.8</td>
<td>1236.6 1.7</td>
</tr>
<tr>
<td>Escherichia coli (ATCC 115575)</td>
<td>28.6 0.6</td>
<td>0.3 0.2</td>
<td>39 0.6</td>
<td>570 0.6</td>
<td>1000 1.6</td>
</tr>
<tr>
<td>E coli + Aeromonas Hybrid</td>
<td>12.0 0.6</td>
<td>1.6 0.4</td>
<td>16.2 0.9</td>
<td>142 0.8</td>
<td>410 1.7</td>
</tr>
<tr>
<td>Candida albicans (ATCC 10231)</td>
<td>11.0 0.9</td>
<td>8.5 0.9</td>
<td>11.5 0.9</td>
<td>705.6 0.3</td>
<td>5000 1.8</td>
</tr>
<tr>
<td>Aspergillus fumigatus Waste water isolate</td>
<td>1.0 0.8</td>
<td>0.0 0.3</td>
<td>3.3 0.9</td>
<td>340 0.7</td>
<td>5000 28.2</td>
</tr>
</tbody>
</table>

**Key:** MO => *Moringa oleifera* seed  
ATCC => American Type Culture Collection  
Values indicated are mean values for three treatments
Appendices

APPENDIX 2: COAGULATION (TURBIDITY REMOVAL) LEVELS FROM SYNTHETIC MODEL TURBID WATER USING MO EXTRACTS PRETREATMENT AND SAND FILTRATION

<table>
<thead>
<tr>
<th>Synthetic Turbid model Bentonite and soil water</th>
<th>Turbidity</th>
<th>PH</th>
<th>Residence time</th>
</tr>
</thead>
<tbody>
<tr>
<td>MO salt extract Sand filtration</td>
<td>16.7</td>
<td>6.8</td>
<td>45min</td>
</tr>
<tr>
<td>MO Sequential Aqueous sand filtration</td>
<td>7.7</td>
<td>6.8</td>
<td>45 min</td>
</tr>
<tr>
<td>MO crude extract sand filtration</td>
<td>18.7</td>
<td>6.8</td>
<td>45 min</td>
</tr>
<tr>
<td>Sand filtration control</td>
<td>37.9</td>
<td>6.8</td>
<td>45 min</td>
</tr>
<tr>
<td>Initial Values from Synthetic model-RO water</td>
<td>851.3</td>
<td>6.8</td>
<td>45 min</td>
</tr>
</tbody>
</table>
## Appendix 3: Coagulation and Disinfection of Synthetic Turbid Water with MO Extracts – Sand Filtration

<table>
<thead>
<tr>
<th>Synthetic Turbid soil water</th>
<th>Total heterotrophic bacterial counts</th>
<th>Total Fungal(yeasts) counts</th>
<th>Turbidity</th>
<th>Residence time</th>
</tr>
</thead>
<tbody>
<tr>
<td>MO Salt extract Sand filtration</td>
<td>85.6</td>
<td>37.6</td>
<td>1.76</td>
<td>45 min</td>
</tr>
<tr>
<td>MO Sequential Aqueous sand filtration</td>
<td>11.3</td>
<td>7.6</td>
<td>1.13</td>
<td>45 min</td>
</tr>
<tr>
<td>MO crude extract sand filtration</td>
<td>84.6</td>
<td>63.3</td>
<td>19.3</td>
<td>45 min</td>
</tr>
<tr>
<td>Sand filtration control</td>
<td>387.0</td>
<td>58.3</td>
<td>31.2</td>
<td>45 min</td>
</tr>
<tr>
<td>Initial values from Synthetic model-RO water</td>
<td>5000</td>
<td>3263.3</td>
<td>654.3</td>
<td>45 min</td>
</tr>
</tbody>
</table>
**APPENDIX 4: COAGULATION AND DISINFECTION OF MODEL SYNTHETIC TURBID WATER WITH MO EXTRACTS SAND FILTRATION**

<table>
<thead>
<tr>
<th>Synthetic Turbid soil water</th>
<th>Total heterotrophic bacterial counts</th>
<th>Total Fungal /yeasts counts</th>
<th>Turbidity</th>
<th>E coli Counts</th>
<th>Aeromonas counts</th>
<th>Res. time</th>
</tr>
</thead>
<tbody>
<tr>
<td>MO Salt extract Sand filtration</td>
<td>114</td>
<td>71</td>
<td>11.7</td>
<td>16</td>
<td>13.6</td>
<td>45min</td>
</tr>
<tr>
<td>MO Sequential Aqueous sand filtration</td>
<td>114.3</td>
<td>41</td>
<td>10.2</td>
<td>1.6</td>
<td>13.3</td>
<td>45 min</td>
</tr>
<tr>
<td>MO crude extract sand filtration</td>
<td>175</td>
<td>92</td>
<td>20.4</td>
<td>15.3</td>
<td>113.6</td>
<td>45 min</td>
</tr>
<tr>
<td>Sand filtration control</td>
<td>669.3</td>
<td>391.6</td>
<td>41.6</td>
<td>428.6</td>
<td>194.6</td>
<td>45 min</td>
</tr>
<tr>
<td>Initial values from Synthetic model-RO water</td>
<td>710,000</td>
<td>5700</td>
<td>3199.3</td>
<td>5866.6</td>
<td>5653</td>
<td>45 min</td>
</tr>
</tbody>
</table>
### APPENDIX 5: TREATMENTS WITH AQUEOUS SEQUENTIAL MO EXTRACT

#### SAND FILTRATION

<table>
<thead>
<tr>
<th>Treatment parameter</th>
<th>Flow rates</th>
<th>Loading rate</th>
<th>Clogging rate</th>
<th>Initial loading volume</th>
<th>Residenc e time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil RO water</td>
<td>0.02ml/sec</td>
<td>200ml/90min</td>
<td>Approx, 24 hours</td>
<td>200 mls, it varies along</td>
<td>45 min</td>
</tr>
<tr>
<td>Bentonite RO water</td>
<td>0.4ml/sec</td>
<td>200ml/45min</td>
<td>-</td>
<td></td>
<td>45min</td>
</tr>
<tr>
<td>E coli RO water</td>
<td>0.3ml/sec</td>
<td>200ml/45min</td>
<td>-</td>
<td></td>
<td>45 min</td>
</tr>
<tr>
<td>Model hybrid RO water, bentonite, soil, Ecoli,Aero</td>
<td>0.1ml/sec</td>
<td>200ml/60min</td>
<td>-</td>
<td></td>
<td>45 min</td>
</tr>
<tr>
<td>Aspergillus RO water</td>
<td>0.2ml/sec</td>
<td>200ml/45min</td>
<td>Approx.18 hrs</td>
<td></td>
<td>45 min</td>
</tr>
</tbody>
</table>
### APPENDIX 6: TREATMENTS WITH MO SALT EXTRACT SAND FILTRATION

<table>
<thead>
<tr>
<th>Treatment parameter</th>
<th>Flow rates</th>
<th>Loading rate</th>
<th>Clogging rate</th>
<th>Initial loading volume</th>
<th>Residence time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil RO water</td>
<td>0.1ml/sec</td>
<td>200ml/80min</td>
<td>Approx, 24 hours</td>
<td>200mls, it varies along</td>
<td>45 min</td>
</tr>
<tr>
<td>Bentonite RO water</td>
<td>0.5ml/sec</td>
<td>200ml/43min</td>
<td>-</td>
<td></td>
<td>45min</td>
</tr>
<tr>
<td>E coli RO water</td>
<td>0.4ml/sec</td>
<td>200ml/40min</td>
<td>-</td>
<td></td>
<td>45 min</td>
</tr>
<tr>
<td>Model hybrid RO water, bentonite, soil, Ecoli, Aero</td>
<td>0.3ml/sec</td>
<td>200ml/55min</td>
<td>-</td>
<td></td>
<td>45 min</td>
</tr>
<tr>
<td>Aspergillus RO water</td>
<td>0.3ml/sec</td>
<td>200ml/42min</td>
<td>-</td>
<td></td>
<td>45 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>45 min</td>
</tr>
</tbody>
</table>
### APPENDIX 7: TREATMENTS WITH MO CRUDE EXTRACT SAND FILTRATION

<table>
<thead>
<tr>
<th>Treatment parameter</th>
<th>Flow rates</th>
<th>Loading rate</th>
<th>Clogging rate</th>
<th>Initial loading volume</th>
<th>Residenc e time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil RO water</td>
<td>0.2ml/sec</td>
<td>200ml/60min</td>
<td>Approx. 24 hours</td>
<td>200mls, it varies along</td>
<td>45 min</td>
</tr>
<tr>
<td>Bentonite RO water</td>
<td>0.6ml/sec</td>
<td>200ml/35min</td>
<td>-</td>
<td></td>
<td>45min</td>
</tr>
<tr>
<td>E coli RO water</td>
<td>0.4ml/sec</td>
<td>200ml/40min</td>
<td>-</td>
<td></td>
<td>45 min</td>
</tr>
<tr>
<td>Model hybrid RO water, bentonite, soil, Ecoli, Aero</td>
<td>0.4ml/sec</td>
<td>200ml/45min</td>
<td>-</td>
<td></td>
<td>45 min</td>
</tr>
<tr>
<td>Aspergillus RO water</td>
<td>0.4ml/sec</td>
<td>200ml/40min</td>
<td>Approx. 18hrs</td>
<td></td>
<td>45 min</td>
</tr>
</tbody>
</table>
**APPENDIX 8: TREATMENT WITH SAND FILTER (SAND FILTRATION) CONTROL**

<table>
<thead>
<tr>
<th>Treatment parameter</th>
<th>Flow rates</th>
<th>Loading rate</th>
<th>Clogging rate</th>
<th>Initial loading volume</th>
<th>Residence time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil RO water</td>
<td>0.4ml/sec</td>
<td>200ml/50mi</td>
<td>Approx, 12 hours</td>
<td>200mls, it varies along</td>
<td>45 min</td>
</tr>
<tr>
<td>Bentonite RO water</td>
<td>0.8ml/sec</td>
<td>200ml/20mi</td>
<td>-</td>
<td></td>
<td>45min</td>
</tr>
<tr>
<td>E coli RO water</td>
<td>0.6ml/sec</td>
<td>200ml/45mi</td>
<td>-</td>
<td></td>
<td>45 min</td>
</tr>
<tr>
<td>Model hybrid</td>
<td>0.5ml/sec</td>
<td>200ml/50mi</td>
<td>-</td>
<td></td>
<td>45 min</td>
</tr>
<tr>
<td>Aspergillus RO water</td>
<td>0.5ml/sec</td>
<td>200ml/40mi</td>
<td>-</td>
<td></td>
<td>45 min</td>
</tr>
</tbody>
</table>
## APPENDIX 9: SYNTHETIC TURBID WATER WITH BENTONITE TREATED WITH CRUDE MO SEED SOLUTION AND ALUM

<table>
<thead>
<tr>
<th>Synthetic turbid water</th>
<th>Turbidity removal by crude MO solution (5ml)</th>
<th>Turbidity removal by crude MO solution (10ml)</th>
<th>Turbidity removal by Alum sulphate solution (5ml)</th>
<th>Turbidity removal by Alum sulphate solution (10ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bentonite spiked water (Approx. 1000NTU)</td>
<td>96% removal in 5 mins</td>
<td>97% removal in 5 mins 99.5% removal in 30 mins</td>
<td>90% removal in 5 mins 99.9% removal in 30 mins</td>
<td>90% removal in 5 mins 99.9% removal in 30 mins</td>
</tr>
<tr>
<td>(300NTU)</td>
<td>96% removal in 5 mins 99% removal in 30 mins</td>
<td>97% removal in 5 mins 99.5% removal in 30 mins</td>
<td>90% removal in 5 mins 99.9% removal in 30 mins</td>
<td>90% removal in 5 mins 99.9% removal in 30 mins</td>
</tr>
</tbody>
</table>
APPENDIX 10: DRYING MORINGA EXTRACTS IN LIQUID NITROGEN

Liquid nitrogen applied to dry off solvents from Moringa extracts after a polarity based cold extraction process.
APPENDIX 11: EXPERIMENTAL RIGS USING FOR WATER TREATMENT

Fabrication of experimental rigs using for water treatment.

Rigs seeded with coliforms synthetic contaminated water
APPENDIX 12: A RANGE OF CULTURE MEDIA USED IN THE EXPERIMENTS

A range of culture media used to grow E.coli, Aeromonas hydrophila, Aspergillus fumigatus and coliforms.
A pump was inserted into the bore hole to pump water into the moringa pre
treatment tank.

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Washing of the filter bed after 5 months.
APPENDIX 14: POSTER PRESENTATION OF THIS RESEARCH DURING THE CHEMICAL ENGINEERING CONFERENCE (CHEMEECA, 2010)

CHEAP ALTERNATIVE BIOCOAGULANT-SAND FILTER SYSTEM FOR WATER PURIFICATION IN LOW INCOME EARNING COUNTRIES

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Introduction
Water is a precious resource for human life; however, its consumption in low-income countries is a major challenge. The problem of clean water is exacerbated in low-income countries, where water is often contaminated with various pathogens and pollutants. This leads to a high incidence of water-borne diseases, which are a significant cause of illness and death, particularly among children. In low-income countries, the demand for clean water is high, but the supply is limited, leading to a significant gap between demand and supply.

Importance of water
Water is a fundamental need for human survival, and its availability is crucial for economic, social, and environmental development.

Problem Analysis 1
Untreated water is a significant health risk in low-income countries. The World Health Organization (WHO) has set standards for various water quality parameters to ensure public health. These standards are designed to protect against water-borne diseases, but in many low-income countries, water quality fails to meet these standards.

Problem Analysis 2
The use of sand filters in water treatment is a cost-effective method that can improve the quality of water. Sand filters are simple to operate and require minimal maintenance. However, the choice of filter media is critical, as the effectiveness of the filter depends on the type of media used.

Significance of this research
This research aims to develop a cost-effective and sustainable water treatment system that can be implemented in low-income countries. The proposed system is based on the use of Moringa plant material as a biocoagulant, which can be harvested locally and is easily accessible in many low-income countries.

Aims/Objectives
The objective of this research is to develop a biocoagulant-sand filter system to treat water. The system is designed to be cost-effective and sustainable, while meeting the WHO standards for water quality.

Materials and Methods
The research involved the development of a biocoagulant-sand filter system. The biocoagulant was prepared from Moringa seeds, which were harvested from a farm in Nigeria. The filter media was composed of sand, which was collected from a local source.

Results
The results show that the biocoagulant-sand filter system is effective in treating water. The system was able to remove turbidity, coliform bacteria, and other contaminants, bringing the water quality to acceptable levels.

Conclusion and Recommendations
The developed system is a cost-effective and sustainable method for water treatment in low-income countries. The system is simple to operate and requires minimal maintenance, making it an ideal solution for low-income countries. Further research is needed to optimize the system and to evaluate its long-term effectiveness.

Acknowledgements
The authors acknowledge the financial support from the Australian Government’s Research Training Program (RTP). The research was also supported by the Phytobiotechnology Research Foundation in Cameroon.

Table 1: Comparison of water quality parameters before and after treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated</th>
<th>Treated</th>
<th>WHO value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turbidity (NTU)</td>
<td>51.1</td>
<td>2.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Coliforms (cfu/ml)</td>
<td>5100</td>
<td>&lt;10</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Total solids (mg/l)</td>
<td>343</td>
<td>20</td>
<td>50</td>
</tr>
</tbody>
</table>

References


Chee Kwei is acknowledged for editing the slides.
APPENDIX 15: RISK ASSESSMENT INVOLVED IN THE RESEARCH WORK ON A SUSTAINABLE LOW-COST PHYTODISINFECTANT-SAND FILTER ALTERNATIVE FOR WATER PURIFICATION

Risk involved with the use of Moringa oleifera seeds.

A risk assessment and Standard operating procedures were carried out before commencement of this research and was approved by the School of Chemical engineering safety officer. *Moringa oleifera* is an edible plant; the leaves contain up to 21% protein and are used in many parts of the world as a vegetable. The seeds, which were used in this research work for water treatment, are rich in oil. The oils have wide culinary and cosmetic applications. Generally no risk was anticipated in handling *Moringa oleifera* seeds. Residues from the seeds after treating water were disposed of safely, like any other organic/domestic waste.

1. The use of Soil.

Fifty (50) grams of soil was used in this experiment to prepare synthetic turbid water containing aerobic mesophilic bacteria. The risk involve with soil is possible microbial contamination of the body. Normally, soil from areas where cattle and poultry faces or from a dumpsite was avoided as this could contain potentially harmful organisms. Moreover, only 50 grams of soil was collected. Protective hand gloves and nose mask were used at all times.

2. Risk Involved with *Escherichia coli*

In this research work, pure culture of *E. coli* was used to prepare contaminated water in the lab. Equally, Moringa seed extracts were tested for its efficacy on *E. coli*. *E. coli* when ingested could cause a range of diseases such as diarrhoeal and urinary tract infections. *E. coli* is not as noxious as other pathogens, rather the term opportunistic pathogen is often referred to *E. coli* infections. The bacterium is a normal flora in the gut of every human being and rather plays a key role in keeping pathogenic bacteria away through the secretion of colicine—an antimicrobial substance. Additionally *E. coli* is one of the most widely studied and frequently used bacterium for lab work in most biotechnological research work. In this
current research work, genetically modified strains of *E. coli* were not used. Strict lab ethics such as putting on lab coats, eye and nose protection as well as putting on hand gloves at all times was applied. Petri dishes with *E. coli* culture were sterilized by autoclaving at 121 degrees C for 15 minutes before disposal. As such the chances of contaminating either the researcher or other lab staff members was minimized.

In summary, Standard microbiological operating procedures such as aseptic techniques as well as standards specified by the Bio safety containment level ([http://bio.research.ucsc.edu/safety/colihaz.html](http://bio.research.ucsc.edu/safety/colihaz.html)) as well as the standard laid out by the Joint FAO/WHO activities on risk assessment of microbial hazards in food ([http://www.who.int/foodsafety](http://www.who.int/foodsafety)) was at all times applied when executing this research task.

4 Risk involved with the use of chemicals

In this study, a number of chemicals were used. For instance, acetone, methanol and other organic solvents were used to extract components from Moringa seeds. This is by far classified as the high risk element so far as this research work is concerned. Dimethysulfoxide, sulphuric acid, and hydrochloric acid are the most noxious chemical which are potential eye and skin irritants. However, in most cases only a few millilitres were required for use in this study. Caution was taken to avoid skin and eye contact by having lab coats, eye and nose protection, hand gloves put on at all times while in the lab. The chemicals were carefully labelled and locked up in a cubicle to avoid anyone accidentally coming in contact. No one was, however, allowed to come around the working station unless on a guided tour with appropriate skin and eye protection put on.

5. Risk involved with the use of glasswares

Glassware was used during this study. These included beakers, conical flasks etc. Cuts from damaged glassware cannot be ruled out. Accidents could occur. However, hand gloves, lab coat and eye protection shall be worn at all times. Glass wares were carefully handled bearing in mind its fragility.