PHYCOREMEDIATION EFFICACY OF *Chlorella vulgaris, Synechocystis salina* AND *Gloeocapsa gelatinosa* ON WASTE WATER FROM COFFEE, TEA AND SUGAR FACTORIES IN BUNGOMA, NANDI AND KAKAMEGA; KENYA.

BY

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DECLARATION

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I, Alexander M Mbeke do hereby declare that this thesis is my original work and has not been submitted for the award of degree or diploma in any other university or college.

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DEDICATION

To my late parents Mr. and Mrs. Job Mbeke for the sacrifice they made for me to get quality education. May their souls rest in peace.

ABSTRACT

The prevention of rivers and other water sources from pollution and the protection of public health by safeguarding water supplies against exposure to pollutants and the spread of diseases are the two major foundamental reasons for treating waste water.The conventional methods of treatment are inefficient, costly, unsustainable, outdated and oftenly results in an effluent heavily loaded with pollutants. Excess nitrates and phosphates causes eutrophication of the receiving water bodies and when taken up by human and animals it may cause food digestion associated diseases and methaemoglobin. Phycoremediation is an alternative way of waste water treatment which involves the use of algae for the removal or biotransformation of pollutants from the waste water. This study was justified because most of the tea, coffee and sugar factories found in Nandi,Bungoma and Kakamega counties don't effectively treat their waste water. The objectives of this study were to determine the phycoremediation efficacy of *Chlorella vulgaris,Synechocystis salina* and *Gloeocapsa gelatinosa* on the physicochemical parameters of coffee, tea and sugar waste water from Nandi,Bungoma and Kakamega counties,and to assess the phycoremediation efficacies of *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa* on nitrates and phosphates in coffee, tea and sugar effluents aganist WHO permissible standards.Purposive and random sampling were used to obtain four replicate samples from the 26 coffee,tea and sugar factories. 10ml of serial dilutes of pure *C.vulgaris, S.salina and G.gelatinosa* in test tubes were mixed with 100ml of the waste water in a beaker from the three types of waste water then incubated at 25^0c and monitored for nutrient absorption which had an effect on the concentrations of TDS,BOD, COD, pH and conductivity levels.The BOD and the COD were determined using the BOD/COD track machine and pH meter for estimation of pH, while the phosphate and nitrate contents were determined using the colorimetric method before and after specific algal inoculation, while STATISTICA V.8.0 was used in data analysis.Results showed significant differences in TDS Phycoremediation of p = 0.00001,p = 0.0000,p= 0.00006 and p = 0.00864,p =0.00260 and p=0.00662 between day 0 and day 5 in tea and sugar effluents for *S.salina,C.vulgaris* and *G.gelatinosa* respectively.While between day 10 and 15 a non significant difference of TDS and conductivity phycoremediation efficacy of p =1.0000 was recorded in coffee, tea and sugar effluents.The phycoremediation efficacy of BOD was significantly different in the sugar effluent only between day 5 and day 10 with p = 0.03066 and p =0.000905 for *S.salina* and *C.vulgaris* respectively. While between day 10 and 15 the BOD and COD phycoremediations were not siginificantly different (p-value=1.0000) in all the effluents.The phycoremediation efficacy of all the species in the three effluents showed an increase in pH levels of the effluents between day 0 and day 5and no effect between day 10 and day 15.The comparison of the phycoremediation efficacy of *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa* in the three effluents showed insignificant difference in phycoremediation of p>0.05 in the physiochemical parameters except for TDS and conductivity with p =0.015 in the tea effluent and p=0.004 for sugar effluent. The phycoremediation efficacy of nitrates, phosphates, COD,BOD, pH TDS and conductivity was in the order of *Chlorella vulgaris> Synechocystis salina >Gloeocapsa gelatinosa*. The phycoremediation of nitrates and phosphorus by *S.salina, C. vulgaris and G.gelatinosa* against the WHO standards (10mg/l,5mg/l) in the tea effluent showed a phycoremediation significant difference of p=0.00001 and a non significant difference in the sugar effluent nitrates of p = 0.082571,p = 0.057716 and p =0.090334 for *S.salina,C.vulgaris and G.gelatinosa* respectively. In conclusion *S.salina, C.vulgaris* and *G.gelatinosa* were all found to be efficacious and therefore should be recommended in public health phycoremediation of coffee, tea and sugar waste water.

TABLE OF CONTENTS

DECLARATION ii

ACKNOWLEDGEMENT iii

DEDICATION iv

ABSTRACT v

TABLE OF CONTENTS vi

LIST OF ABBREVIATIONS AND ACRONYMS ix

OPERATIONAL DEFINATIONS x

LIST OF TABLES xi

LIST OF FIGURES xii

LIST OF PLATES xiv

LIST OF APPENDICES xv

CHAPTER ONE: INTRODUCTION 1

1.1 Background Information 1

1.2 Historical Cause Leading to the Problem 7

1.3 Problem Statement 8

1.4 Significance of the Study 9

1.5 Justification of the study 10

1.6 Objectives 10

1.6.1 General Objective 10

1.6.2 Specific Objectives 11

1.7 Null Hypothesis 11

1.8 Conceptual Theory 11

CHAPTER TWO: LITERATURE REVIEW 13

2.1 Determination of the phycoremediation efficacies of on the physicochemical parameters of coffee, tea and sugar effluents 13

2.1.1 Comparison of the phycoremediation efficacies of *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa* in coffee, tea and sugar effluents 20

2.2. Phycoremediation efficacies of *Chlorella vulgaris*, *Synechocystis salina* and *Gloeocapsa gelatinosa* on nitrates and phosphates in coffee, tea and sugar effluents aganist WHO permissible stds. 24

CHAPTER THREE: MATERIALS AND METHODS 28

3.1 Study Design 28
3.2 Study Site 28
3.3 Inclusion and Exclusion Criteria 34
3.3.1 Inclusion Criteria 34
3.3.2 Exclusion Criteria 34
3.3.3 Sampling Method 34
3.3.4 Sample Size Determination 35
3.4 Experimental Layout 36
3.5 Determination of the phycoremediation efficacy of *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa* on the physicochemical parameters of coffee, tea and sugar effluents. 37
3.5.1 Preferentail Hydrogen Analysis (pH) 37
3.5.1.1Calibration of the electrode 38
3.5.2 Conductivity Measurement 38
3.5.3 Biological Oxygen Demand (BOD5) 39
3.5.4 Chemical Oxygen Demand (COD) 39
3.5.5. Total Dissolved Solids (TDS) 41
3.6.0 Assement of Phycoremediation efficacies of *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa* on the nitrates and phosphates in tea, coffee and sugar effluents aganist WHO permissible stds. 42
3.6.1 Nitrates (NO3-) according to cadmium reduction method 42
3.6.2 Phosphates (PO4-) analysis according to Andrew *et al.,* (1995). 45
3.6.3 Neubauer chamber method of cell enumeration according to APHA 2005; 46
3.7.0 Comparison of the phycoremediation efficacies of the three algal species in the tea, sugar and coffee effluents. 47
3.8 Data validility and reliability. 50
3.9 Data management and statistical analysis 50

CHAPTER FOUR: RESULTS 51

4.1 Phycoremediation efficacy of *C.vulgaris, S.salina* and *G.gelatinosa* on the physico-chemical parameters of coffee, tea and sugar effluents 52
4.1.1 Comparison of the phycoremediation efficacies of S.salina, C.vulgaris and G.gelatinosa in the coffee, tea and sugar 79
4.2 Phycoremediation efficacy on nitrates and phosphates in the tea, coffee and sugar effluents against WHO permissible stds. 82

CHAPTER FIVE: DISCUSSION 85

5.1 Phycoremediation efficacy of *C.vulgaris, S.salina* and *G.gelatinosa* on the physicochemical parameters of coffee, tea and sugar effluents 85
5.1.1 Comparison of the phycoremediation efficacies of *S. salina, C.vulgaris* and *G.gelatinosa* in tea, sugar and coffee effluents. 92
5.2 Phycoremediation efficacy of *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa* on nitrates and phosphates in tea, coffee and sugar effluents against WHO permissible stds 94

CHAPTER SIX: SUMMARY OF FINDINGS, CONCLUSION AND RECOMMENDATION 98

6.1: Summary of findings; 98
6.2 Conclusion 99
6.3 Recommendations 100
6.4 Recommendation for further studies 100

REFERENCES 101

APPENDICES 111

LIST OF ABBREVIATIONS AND ACRONYMS

A.S.L	Above Sea Level
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
CTA	Coffee and Tea Authority
DO	Dissolved Oxygen
EMCA	Environmental Management and Co-ordination Act
EMF	Electromotive Force
IAPS	Integrated algal pond system
ICO	International Coffee Organization
ITC	International Trade Center
KPLC	Kenya power and lighting company
LVEMP	Lake Victoria Environmental Management Programme
NEMA	National Environmental Management Authority
NO_2^-	Nitrites
NO_3	Nitrates
NTU	Nephelometric Turbidity Unit
PO_4^-	Phosphates
STDS	Standards
TDS	Total Dissolved Solid
TSS	Total Suspended Solid
WARMA	Water resources management authority.
WEF	Water Environment Federation
WSPs	Waste water treatment in Waste Stabilization Ponds
µS/Cm	micro Seimens per centimeter
EMF	Electromotive Force
STDS	Standards.

OPERATIONAL DEFINATIONS

Biodegradation: Chemical process in which materials are consumed by microorganisms and turned into compounds that are natural.

Effluent: Waste water or liquid waste discharged into lake or sea.

Eutrophication: Enrichment of water by nutrients salts that causes structural changes to the ecosystem such as increased production of algae and aquatic plants, depletion of fish species and general deterioration of aquatic biodiversity.

Methemoglobin: Blood disorder in which too little oxygen is delivered to ones cells

Organic Enrichment: Particulates or dissolved matter with dissolved nutrients like nitrates from waste water discharge.

Phycoremediation: The use of micro algae for the removal or biotransformation of pollutants including nutrients and xenobiotics from waste water and Co_2 from waste air.

Phytoremediation: Is the direct use of living green plants for insitu, or in place removal, degradation or containment of contaminants in waste water.

Xenobiotics: Substances that are present in much higher concentrations than are usual.

LIST OF TABLES

Table 4.1 Deviations of Effluent and Influent Physicochemical Parameters in coffee, sugar and tea 51

Table 4.2 Phycoremediation efficacies of *C.vulgaris, S.salina* and *G.gelatinosa* on the TDS of coffee, tea and sugar effluent 53

Table 4.3 Phycoremediation efficacies of *S.salina, C.vulgaris* and *G.gelatinosa* on the pH of coffee, tea and sugar effluents. 57

Table 4.4 Phycoremediation efficacies of *C.vulgaris, S.salina* and *G.gelatinosa* on the nitrates of coffee, tea and sugar effluent 60

Table 4.5 Phycoremediation efficacy of *C.vulgaris, S.salina* and *G.gelatinosa* on the phosphates of coffee, tea and sugar effluent 64

Table 4.6 Phycoremediation efficacy of *C.vulgaris, S.salina* and *G.gelatinosa* on the BOD of coffee, tea and sugar effluent 69

Table 4.7 Phycoremediation efficacy of *C.vulgaris, S.salina* and *G.gelatinosa* on the COD of coffee, tea and sugar effluent 72

Table 4.8 Phycoremediation effect of *C.vulgaris, S.salina* and *G.gelatinosa* on the conductivity of coffee, tea and sugar effluent; 77

Table 4.9 Comparison of phycoremediation efficacies in coffee effluent (ANOVA) 80

Table 4.10 Comparison of phycoremediation efficacies in tea effluent (ANOVA) 81

Table 4.11 Comparison of phycoremediation efficacy in sugar effluent (ANOVA) 82

Table 4.12 Phycoremediation efficacies on nitrates in coffee,tea and sugar effluents against WHO stds 83

Table 4.13 Phycoremediation efficacies on phosphates in coffee, tea and sugar effluents against WHO stds 84

LIST OF FIGURES

Figure 1.1: Conceptual frame work (Andersen 2012) 12

Figure 4.1 Phycoremediation efficacies of *C .vulgaris, S.salina* and *G.gelatinosa* on TDS of Tea effluent 54

Figure 4.2 Phycoremediation efficacies of *C .vulgaris, S.salina* and *G.gelatinosa* on TDS of sugar effluent. 55

Figure 4.3 Phycoremediation efficacies of *C .vulgaris, S.salina* and *G.gelatinosa* on TDS of Coffee effluent. 55

Figure 4.4 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on pH of coffee effluent 57

Figure 4.5 Phycoremediation efficacy of *C .vulgaris, S.salina*, and *G.gelatinosa* on pH of tea effluent 58

Figure 4.6 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on pH of sugar effluent 58

Figure 4.7 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on nitrates of coffee effluent 61

Figure 4.8 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on nitrates of tea effluent 61

Figure 4.9 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on nitrates of sugar effluent. 62

Figure 4. 10 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on phosphates of coffee effluent 65

Figure 4. 11 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on phosphates of tea effluent 66

Figure 4.12 Phycoremediation efficacy of *C.vulgaris, S.salina* and *G.gelatinosa* on phosphates of sugar effluent 67

Figure 4. 13 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on BOD of coffee effluent 69

Figure 4.14 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on BOD of tea effluent 70

Figure 4.15 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on BOD of sugar effluent. 70

Figure 4.16 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on COD of coffee effluent 73

Figure 4.17 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on COD of tea effluent 74
Figure 4.18 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on COD of sugar effluent 75
Figure 4.19 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on Conductivity of coffee effluent 78
Figure 4.20 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on Conductivity of tea effluent..... 78
Figure 4.21 Phycoremediation efficacy of *C.vulgaris, S.salina* and *G.gelatinosa* on Conductivity of sugar effluent..... 79

LIST OF PLATES

Plate 3.1: Bungoma County 0.84790N,34.70200E (Mt. Elgon sub county) coffee factories (courtesy of KPLC 2015) 29

Plate 3.2: Nandi County 0.10360N, 35.17770E; Tea factories (courtesy of KPLC 2015). 30

Plate 3.3: Nandi County 0.10360N,35.17770E; Tea factories (courtesy of KPLC 2015). 31

Plate 3.4: Kakamega County 0.28370N, 34.7515 0 E; Western Sugar company in (courtesy of KPLC 2015). 32

Plate 3.5: Kakamega County 0.2837 0N, 34.7515 0 E; Butali Sugar company in (courtesy of KPLC 2015). 33

Plate 3.6: Waste water samples incubated in a COD reactor for two hours and the BOD trak bottles (photograph taken on 18.6.2015; by A. Mbeke). 40

Plate 3.7: Electrocoagulation experimental method in progress. (photograph taken on 6.5.15 by A. Mbeke) 43

Plate 3.8: A sketch diagram of the Electrocoagulation method. 43

Plate 3.9: Phosphate, nitrate and COD estimation using a spectrophotometer 45

Plate 3.10: Waste water from different sampled locations inoculated with the specific algal species before incubation at the laboratory conditions. 47

Plate 3.11: *Chlorella vulgaris* microscope photographs (21.6.15 by A.Mbeke) 48

Plate 3.12: *Gleocapsa gelatinosa* microscope photographs (21.6.15 by A.Mbeke) 49

Plate 3.13: *Synechocystis salina* microscope photograph x45 (21.6.15 by A.Mbeke) 49

LIST OF APPENDICES

APPENDIX I: APPARATUS, EQUIPMENT AND REAGENTS USED IN pH ESTIMATION.....111

APPENDIX II: APPARATUS, EQUIPMENT AND PROCEDURE FOR CONDUCTIVITY.....112

APPENDIX III: CHEMICAL OXYGEN DEMAND BY REFLUX METHOD APPARATUS AND REAGENTS;.....113

APPENDIX IV: APPARATUS AND EQUIPMENT USED IN TOTAL DISSOLVED SOLIDS DETERMINATION.....114

APPENDIX V: NITRATE ESTIMATION APPARATUS AND EQUIPMENT 115

APPENDIX VI: RESEARCH PERMIT NANDI COUNTY.....116

APPENDIX VII: RESEARCH PERMIT KAKAMEGA COUNTY.....117

APPENDIX VIII: RESEARCH PERMIT BUNGOMA COUNTY.....118

APPENDIX IX: RESEARCH PERMIT ELDORET WATER & SANITATION COMPANY.....119

APPENDIX X: SIGNIFICANCE OF PHYCOREMEDIATION EFFICACY BETWEEN DAYS (T-TESTS).....120

APPENDIX XI: INFLUENT AND EFFLUENT OF PARAMETERS FROM DIFFERENT SITES.....135

CHAPTER ONE: INTRODUCTION

1.1 Background Information

The majority of waterborne microorganisms that are known to cause disease come from organic pollutants found in waste water. Microbial pathogens are therefore critical factors contributing to numerous waterborne disease outbreaks. Diseases caused by bacteria,viruses and protozoa are the most common public health hazards associated with untreated drinking and recreational waters. Contaminated tea, coffee and sugar waste water is therefore a vehicle for several waterborne diseases such as cholera, shigellosis, typhoid fever, campylobacteriosis, Hepatitis, salmonellosis and giardiasis (WHO, 2006). Microbial pathogens found in these effluents can also cause chronic diseases with costly long term effects which have been studied and documented, such as degenerative heart disease and stomach ulcers (WHO, 2006).

Phycoremediation is therefore defined as the use of macroalgae or microalgae for the effective removal or biotransformation of pollutants, including nutrients and xenobiotics of public health importance from waste water and CO_2 from waste air (Olguin 2003). Phycoremediation therefore is applied in the remediation of environmental pollutants using algae. Globally all issues related to the release of contaminated water to the environment needs to be addressed because of the negative public health consequences manifested within the environment (Rawat *et al*., 2011). Human population appears to be the biggest casualties due to the decline of the availability of the sustainable fresh water sources leading to the emergence of water associated diseases within the communities where these waste waters are released into (Sood *et al.,* 2012).

The nitrogen and phosphorous are the two major chemical contaminants in agricultural waste water (Larsdotter, 2006). The presence of nitrogen in waste water discharge can have undesirable public health impacts. Organic nitrogen, ammonium (NH_4^+ or NH_3), nitrite (NO_2) and nitrate (NO_3) are the principal forms of nitrogen (Zhao *et al*., 2016). In untreated waste water nitrogen is usually in the form of ammonia and organic nitrogen, both soluble and particulate (Larsdotter, 2006). The occurrences are generally associated with disposal of agricultural effluents and fertilizer application to the coffee, tea and sugar agricultural crops. The dangers that all these incidents associated with the chemical compound have posed are a clear indication that nitrogen nutrients must be removed from waste water before discharge (Zhao *et al*., 2016).

Methemoglobinemia is the most significant public health problem associated with nitrate in water which when in excess it's normally converted into nitrite. Nitrite pollutants alter the normal form of haemoglobin, which carries oxygen molecule in the red blood cells to the rest of the human body, into methemoglobin form that cannot carry the oxygen molecule (Richmond, 2004). Similarly, nitrogen in the form of ammonia is toxic to fish and exerts an oxygen demand on receiving water by nitrifiers (Jenkins *et al.,* 2003).Thus high concentrations of nitrate discharged into river bodies and eventually taken up for drinking can be converted into nitrites which can cause a temporary blood disorder known as methemoglobinemia that can cause suffocation and finally death (Olguin, 2003). Nitrates and nitrites are also of concern because nitrites react with amino acids in the stomach to form nitrosamines which have been found to be powerful carcinogens of considerable public health concern (Olguin, 2003). But since nitrate cannot be completely removed from water, techniques for decreasing the nitrates to allowable limits which can be tolerated in drinking water of <10mgl needs to be put in place (WHO).The study set out to accomplish this through phycoremediation.

Naturally surface waters contain levels of phosphorus in various compounds, which is an essential constituent of living organisms. In natural conditions, phosphorus concentration in fresh waters is balanced; however when phosphorus input to water bodies is higher than it can be assimilated by living organisms, like in the case of phosphate loaded agricultural effluent, the problem of excess phosphorus content occurs (Rybicki, 1997). Since phosphate is the limiting component for growth in most ecosystems and emission of phosphate in surface waters, this has lead to eutrophication and algal bloom, thus having negative impacts on nature conservation, recreation and drinking water production. It is therefore necessary to control the emission of phosphates from discharges of waste water (van Larsdrecht, 2005).

The excess content of phosphorus in receiving waters usually leads to eutrophication, altered natural composition and species diversity of aquatic communities, impairs recreational values of surface waters, impede commercial fishing and pose public health problems for water treatment (Olguin, 2003).When deprived of oxygen, fishes and other aquatic organisms die, emitting foul odors which are of public health concern. Controlling phosphorus discharge in agricultural and industrial waste water treatment plants is thus a key factor in preventing eutrophication of surface waters (Olguin, 2003).

Also, the presence of nitrogen and phosphorus nutrients in fresh water bodies can cause conditions that favour the growth of toxin-producing cyanobacteria and algae. The resulting toxins can cause adverse public health problems like gastroenteritis, liver damage, nervous system impairment, skin irritation and even liver cancer especially in animals (WHO, 2006). Public health problems associated with cyanotoxins have been documented in several countries, including Australia, Brazil, Canada, China, the United Kingdom, the United States of America and Zimbabwe (WHO, 2006).

Currently, the significance of the presence of fresh water is wildely accepted and appreciated and this has lead to the re-emergence of an increased campaign on the need of having increased and sustainable surface fresh water coverage (Horan, 1990). Many forums within the world have therefore not shied away from aggressively advocating for the establishment of water resource management bodies with the aim of safe guarding the quality and sustainability of fresh water sources (Lim *et al.,* 2010).The rural to urban migration in most of the developing counties and the agricultural mechanization within some counties has also lead to the scramble for the scarce fresh water sources. Increased number of factories due to agricultural mechanization has lead to alot of waste water and other environmental pollutants discharged to the nearby freshwater sources (De la and Pauw, 1988).Since pollution is not natural but rather a man-made phenomenona a lot of organic and inorganic xenobiotic substances have been released into the nearby ecosystem mainly from agricultural, industrial and domestic water related activities (Lim *et al.*,2010).The xenobiotics have lead to organic and inorganic contamination which must be addressed urgently by way of exploring waste water recycling feasibilities and other recoveries strategies (Mouchet,1986).Through Phycoremediation however these contaminants are likely to be reduced into acceptable levels thereby addressing some of the waterborne diseases and other public health issues which may arise from the domestic use of the contaminated effluents (Mouchet,1986).

Coffee, tea and sugar are some of the few cash crops which have continued to earn Kenya foreign exchange for many years and the economy of Kenya has greatly been driven by the three crops. Agricultural industries plays avery pivotal role in the economic growth and development of any country, but of great concern are the effluents generated and released by these factories which normally have a high degree of organic pollutants harmful to human and the aquatic ecosystem (Lim *et al.,* 2010).They also alter the physicochemical

characteristics of the nearby fresh water bodies thereby introducing an ecological imbalance of the fauna and flora and possing a great public health concern (Akali *et al.,* 2011).

The tea, sugar and coffee effluents released into the nearby fresh water sources are known to bring about eutrophication a public health environmental nuisance which may deprive oxygen to the fish and other aquatic organisms leading to high fish mortality and eventual emission of foul odours which are of public health interest to the human beings. The decreased aquatic biodiversity therefore have an effect on the economy and public health of human and animals who wholly depend on the streams and rivers where these factories discharge their effluents into (Ayyasamy *et al.,*2008).The factory effluents also have pollutants with unpleasant odour and colour which when released into the receiving water bodies are known to bring about high oxygen demands leading to an eventual reduced oxygen supply and this condition inturn changes the pH ,BOD, COD, TDS and conductivity characteristics of the receiving water bodies (Sydney *et al.,*2011).

It has been noted that the conventional ways of waste water treatment are inadequate in ensuring a sustainable safe water supply and a proper waste water disposal mechanism for the general public (Hongtao *et al*., 2013).To address this challenge therefore, Hongtao *et al*., (2013) concluded that collaborations are needed into transforming to green economy, devising innovative technologies, geared towards improving operations, maintenance and algal energy harvesting which can be made possible through phycoremediation processes of the waste waters. It is a requirement in Kenya for all industries generating waste water to harvest it and subject it to a treatment process before discharge to the nearby rivers and streams. Sanitary conditions are therefore a mandatory requirement for all industries in order to deter the occurrence of any waterborne diseases and other unwanted public health conditions likely to be deleterious to general public (Ayyasamy *et al.,*2008). The release of deleterious and injurous materials into the fresh water resources is also highly discouraged and that is why every one living in Kenya has aduty to create and maintain a healthy environment through the provisions contained in the Environmental Management and Co-ordination Act of 1999. Lake Victoria Environmental Management Programme (LVEMP) and the general public over the years have raised a lot of serious public health concerns on the increased pollution of rivers passing through the tea, coffee and sugar factories and discharging into Lake Victoria (Maghanga *et al.,* 2009).

Aerobic or anaerobic biological degradation of waste water does not ensure the sufficient removal of inorganic pollutants such as nitrates, ammonia and phosphates ions, which in turn may cause over-fertilization of water bodies causing harmful micro-macroalgal blooms (Becker, 1994).Therefore further treatment is always required inorder to mitigate the probable effects of over-fertilization where phosphates and nitrates are considered to be the key causes (Akali *et al*., 2011).

Microalgae were the first photosynthetic microorganisms to colonize the earth and this was due to their photosynthetic ability and an overall effect of increasing the oxygen levels within the atmosphere (Olguin.,2003).The growth of these micro-macroalgae within the ecosystem is therefore dependent on the availability of carbon, nitrogen and phosphorous compounds as well as other essential trace elements within the environment and in turn the algae enrich the environment with oxygen derived from the photosynthetic process (Huppe and Turpin., 1994). Algae can therefore grow in diverse environments and ecological zones and ecosystems such as on soils, fresh and marine environments, and even in waste water streams from various different sources (Olguin., 2003).The type of algae growing on a particular aquatic environment on the other hand depends on the presence or absence of a given organic waste within that environment (Murali and Nisha., 2009).Therefore the presence of certain species of algae can be used as an indicator of pollution while others can indicate fresh water purity (Oilgae.,2009; Benemann *et al.,* 2002;Sawayama, *et al*., 2000).

The micro-macroalgal study and propagation for use in waste water phycoremediation in a symbiotic consortium with bacteria began in ernest in 1950s in the photosynthetic treatment of domestic waste water in America.The holding and stabilization ponds used the photosynthetic algae to absorb all the nutrient pollutants in the waste water and also in providing oxygen to the aerobic bacteria for the biodegradation of the organic pollutants.The bacteria in return released carbon dioxide (CO_2) which was used during photosynthesis process by the algae, and this clearly improved the chemical profile and the physical properties of the waste water (Oswald *et al.,* 1957).The stabilization ponds have now been replaced by a system with enhanced oxygen supply and an improved algal reactor known as high rate algal ponds oxidation system (HRAP). The HRAP provides a better method of waste water treatment capable of phycoremediating large volumes of waste water because of the decreased retention period. However the intense algal photosynthesis is responsible in providing a lot of saturated oxygen needed to drive the aerobic treatment process and

assimilation of waste water nutrients into algal biomass (Oilgae., 2009; Tebbutt., 1983; Horan., 1990; Lim *et al.*, 2010).

The increased algal use in waste water treatment has also evoked the potential for mass production of high algal biomass produced during phycoremediation process in the algae inoculated ponds and also during other algae application endevours (Wang *et al.*, 2010; Chinnasamy *et al*., 2010; Zhou *et al.*, 2011; Zhou *et al.*, 2012a).Waste waters provides the required nutrients suitable for the propagation of microalgae as evidenced by the high algal productivity and growth rates and also the high algal nutrient removal efficiency which is highly dependent on some abiotic factors such as pH (Zhou *et al*., 2012a; Gray., 1989). High pH can easily remove ammonia nutrients through stripping while phosphorous are removed through coagulation and precipitation technologies (Tam&Wong.1989; Li *et al*.,2011;Zhou *et al.*, 2012a; Zhou *et al.*, 2012b).

Agricultural industries in Africa includes mainly the tea industries, sugar industries, coffee industries, textile, dairy, and paper industries which are known to produce large quantities of waste water with high degrees of pollutant contents (UNEP, 2010; Li *et al.*, 2011;Omosa *et al.*, 2012; Wang *et al.*, 2009). Industrial waste water however has a unique physicochemical nature brought about by the presence of heavy metals which normally presents a big challenge especially in its treatment and the focus is always on removal of the chemical toxins and nutrients through phycoremediation process rather than algal biomass accumulation (Richmond, 2004).Some studies though, however have shown the possibility of algal biomass production through phycoremediation of some selected industrial effluents (Chinnasamy *et al.*, 2010; Hodaifa *et al.*, 2008).

Phycoremediation is therefore an alternative means of bioremediating excess nutrient pollutants found contaminating most of the agricultural waste water.Through phycoremediation, xenobiotics are biotransformed and the waste water made ideal for habitation and use by aquatic animals and human beings and also recycled back for use by the same factories for production process (Olguin, 2012). Phycoremediation studies have always employed the use of different algal species in the bioremediation of various agricultural, industrial, domestic and municipal waste waters, and this is because different species thrives best in different environmental conditions. Phycoremediation technology therefore offers a sustainable, cost effective and environmental friendly way of bioremediating waste water pollutants capable of also producing high algal biomass unlike the conventional technologies

which have likelihoods of secondary pollutions, high costs of operations and also the incomplete utilization of the natural resourses and an overall public health burden brought about by the potential waterborne diseases (Sengar *et al*., 2011;Martinez *et al*., 2000; Lim *et al*.,2010).

Large-scale phycoremediation of industrial, agricultural, domestic and municipal effluent has been done and reported successfully in different parts of the world through studies by Sivasubramanian *et al*., (2009); EL-Sheekh *et al.,* (2015); Sivasubramanian, (2010) and Rao *e t al.,* (2011) who recorded phycoremediation success of 85%,90%,88% and 75% respectively using different micro algae species which were able to remove nutrients very rapidly under laboratory conditions. However phycoremediation studies by Shi *et al.,* (2007) found a high phycoremediation efficiency in the removal of nitrate than that of the phosphate whereas Colak and Kaya (1998) reported a better removal of the phosphorous than the nitrates and this clearly showed that the phycoremediation of waste water is highly dependent on the biotic and abiotic factors and the effluent type.

Wells *et al.,*(2013) in a study done in south Africa suggested that phycoremediation technology through the use of Integrated algal pond system (IAPS) was able to effectively treat waste water and thus providing a sustainable solution to the waste water management problem.Raburu and Okeyo (2000) in their studies on the impact of agro-industrial activities on the quality of river Nyando on the Lake Victoria basin Kenya, found out that nutrient pollutants from the agricultural industries were responsible for the river pollutions and the subsequent waterborne diseases of public health concern thus suggested if phycoremediation was to be employed through IAPS then public heath pollutants in most of the effluents could be reduced to acceptable levels thus protecting the public from most of the pollutants.

1.2 Historical Cause Leading to the Problem

Most tea factories from the study area are known to release copious amounts of contaminated waste water therefore exposing a large number of a population to a variety of waterborne diseases. Since water is a scarce commodity the factories will always find themselves sharing a common fresh water source for their operations with the surrounding community. Once the water is used in the operations of the factories the waste water is always discharged back to the same fresh water rivers where the nearby communities leaving down stream becomes the biggest casualties as they use the nutrient contaminated water for their various domestic purposes together with their animals. The situation is no better in the sugar and coffee

planting areas with the problem becoming worse during the dry seasons with minimal dilution of the waste water or no dilution at all. A lot of concerns therefore have been registered on this problem by Lake Victoria Environmental Management Programme and the communities residing down stream of the factories through the various county public health officers (Maghanga *et al*., 2009). However the adoption of the use of phycoremediation strategies if embraced by these factories can indeed be the solution to the uncontrolled release of these waste waters and if fully adopted it will be able to offer a sustainable method able to address any public health problem resulting from the waste water use.

1.3 Problem Statement

The prevention of rivers and other water sources from pollution and the protection of public health by safeguarding water supplies against the spread of diseases are the two major foundamental reasons for treating waste water. Lack of waste water treatment ponds in the study areas, and where available, the poor design of the tea, coffee and sugar waste water treatment ponds which are often overloaded with pollutants in form of sludge, coupled with the frequent utilization of unskilled labour and lack of capacity to establish the quality of the physicochemical parameters has lead to the continuous discharge of contaminated effluent to the nearby rivers and streams with complete disregard of the possible public health effects. Due to lack of appropriate technologies in the treatment of the contaminated effluents the coffee, tea and sugar factories have continuously been forced to pay to the national environmental management authority a lot of monies each time when they have been found discharging the contaminated effluents to the nearby rivers. The notable contaminants in these effluents were mostly phosphates and nitrate chemicals which most of the farmers use in the growth of these crops. However when these chemical substances find their way to the nearby rivers, their concentration in water increases and may require removal through phycoremediation and assessment against the WHO permissible standards before they become a matter of public health concern,a research gap which this study tried to bridge through the study of phycoremediation efficacy of *Chlorella vulgaris, Synechocystis salina and Gloeocapsa gelatinosa* on the physicochemical parameters of coffee,tea and sugar effluents and also through the assessment of phycoremediation efficacy of *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa* on the nitrates and phosphorous of coffee,tea and sugar effluents against the WHO standards.

1.4 Significance of the Study

The study is significant because the polluted effluents if discharged into the nearby rivers and streams the health of the public will be affected both in terms of food and water availability as well as loss of recreational spots for the public. Increased BOD and COD due to the presence of the pollutants in the waste water will lead to enhanced fish mortality which will have a direct impact on the public heath of the nearby communities whereas increased nitrates and phosphates concentrations discharged into the rivers will lead to a decline in recreational activities due to the accumulation of the algal blooms on the water surface. High phosphates and nitrates discharged into the rivers will also lead to public health problems mainly digestive for high phosphates and metheamoglobin a serious blood disorder associated with high nitrates in water of poor quality. Despite the notable public heath concerns, many studies have laid more emphasis on determination of pollutant levels rather than their removal to acceptable WHO standards a research gap which this study endevoured to bridge. Chemical and mechanical methods of clean up have proved expensive and destructive to the ecosystem, whereas some biological methods used are not sustainably viable (Maghanga *et al.,* 2009). Phycoremediation is therefore a sustainable, eco-friendly method and a suitable biosorbent process more effective at decontaminating the waste waters.

Nandi, Kakamega and Bungoma`were used as the appropriate sampling frames from where representative samples were drawn from.These sampling frames were chosen because Nandi has seventeen tea factories, Kakamega with three sugar factories whereas Bungoma had over sixteen coffee factories. The factories within these three counties are therefore known to discharge their waste water directly to the nearby rivers and streams thereby causing a lot of environmental and public health concerns from most of the public health officers and environmental stakeholders. It was on this background that the study was conducted to establish phycoremediation efficacy of *C.vulgaris, S.salina and G.gelatinosa* on physicochemical parameters of waste water from coffee, tea and sugar factories and also establish the phycoremediation efficacy of *C.vulgaris, S.salina and G.gelatinosa* on nitrates and phosphates in coffee,tea and sugar waste water against WHO permissible standards. The specific nutrients notably the phosphates and nitrates levels had to be determined because of their public heath effects mainly eutrophication and human digestion problems for the case of the phosphates and the conversion of nitrates to nitrites which has the ability to convert haemoglobin to methaemoglobin a temporary blood disorder in humans especially when

nitrates are in high levels in water bodies an aspect which most studies have failed to research on.

1.5 Justification of the study

The study was justified inorder to protect the public from the possible exposure to pollutants found in the poorly treated or untreated tea, coffee and sugar waste water which may have adverse public health effects thereby promoting the public health of the nearby communities and also to providing a solution of the waste water management.Tea, coffee and sugar factories found in Nandi, Bungoma and Kakamega counties have continued to discharge the untreated waste water into the nearby rivers therefore impacting negatively on the public health of the nearby communities. The impacts of the river degradations may result in the decreased levels of oxygen, also the released inorganic substances from the tea, coffee and sugar waste water may accumulate and become biomagnified in the acquatic life therefore becoming a major public health concern both for the local community and even the national and international communities especially when the fish are to be exported. The study is also justified because if the discharged waste water is properly treated then recreational activies within the receiving water bodies will increase thus contributing positively to the public health and economy of the nearby communities and that of the country at large.

The use of three effluents also gave a relationship of the nature of pollutants in most of the agricutural effluents and also made it possible for the researcher to explore on the Phycoremediation efficacy of different algal species with different functional groups namely *C.vulgaris, S.salina* and *G.gelatinosa* a research gap which has not been explored by many researchers.

1.6 Objectives

1.6.1 General Objective

To determine the phycoremediation efficacy of *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa* on waste water from coffee, tea and sugar factories from Bungoma, Nandi and Kakamega counties.

1.6.2 Specific Objectives

1. To determine the phycoremediation efficacy of *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa* on the physicochemical parameters of coffee, tea and sugar effluents from Bungoma, Nandi and Kakamega.
2. To assess the phycoremediation efficacies of *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa* on nitrates and phosphorous in coffee, tea and sugar effluents aganist WHO permissible standards.

1.7 Null Hypothesis

1. There was no relationship between the phycoremediation efficacy of *Chlorella vulgaris, Synechocystis salina and Gloeocapsa gelatinosa* on the physicochemical parameters of coffee, tea and sugar effluents from Bungoma, Nandi and Kakamega.
2. There was no relationship in the assessment of phycoremediation efficacy of *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa* on the nitrates and phosphates in the coffee, tea and sugar effluents against WHO permissible standards

1.8 Conceptual Theory

The study adopted a conceptual frame theory from Andersen (2012) whereby the physicochemical parameters of the effluents from the tea, coffee and sugar factories mainly the BOD, COD,TDS,conductivity, nitrates, phosphates and pH concentration levels were all determined as the variables. After determination the values were kept as the controls of each variable before inoculating a set of each effluent from the three study sites with the respective specific algal cells namely *Chlorella vulgaris, S.salina and G.gelatinosa* which were non variable parameters in the mixture. Incubation of the mixtures were done at 25^0C for upto 15 days with readings on each variable being determined at an interval of five days. The variables were then recorded at day 5 day 10 and day 15 since day zero was held as the control of each variable parameter as shown in Fig1.1.

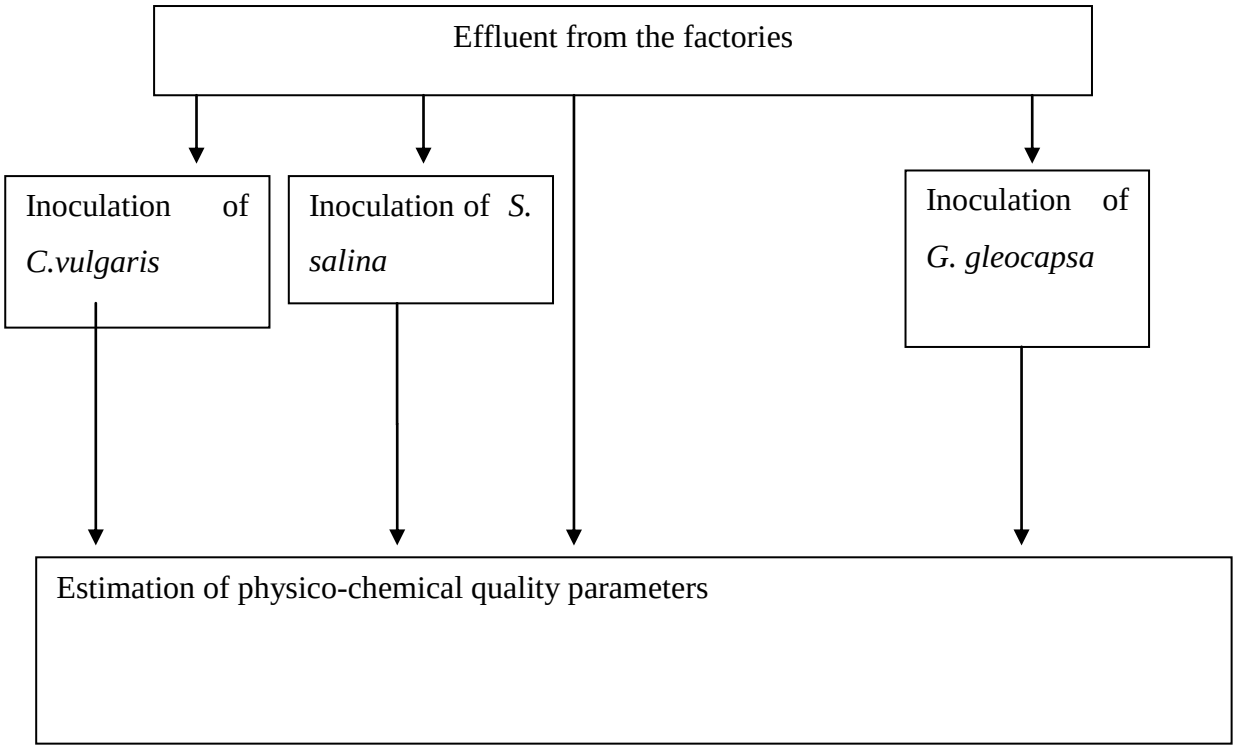


Figure 1.1: Conceptual frame work (Andersen 2012)

CHAPTER TWO: LITERATURE REVIEW

Introduction

Most of the waterborne diseases of public health importance are caused by contaminated effluents discharged into water bodies. Algae are important microorganisms able to remove these contaminants. Therefore primary producers in all kinds of water bodies are known to be micro-macroalgea and most of them are normally involved in water pollution through a variety of important ways. Significantly of all is the enrichments of the receiving water bodies through organic effluents and this may selectively stimulate the growth of algal species producing large surface growths or 'blooms' that in turn lower the water quality thus affecting its use. However, certain algae are known to flourish in polluted water with organic wastes and this plays an important role in "self-purification of thewater bodies" through phycoremediation process (Kotteswari *et al.,* 2007).

In this chapter, the state of knowledge about the pollutants under study is explored. The chapter also reviews various previous phycoremediation studies used in waste water treatment methods

2.1 Determination of the phycoremediation efficacies of *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa* on the physicochemical parameters of coffee, tea and sugar effluents.

Physicochemical parameters in waste water are varied and are known to affect their abiotic characteristics. Some of the major parameters include COD, BOD, pH and TDS. The elevation of the above parameters can be used to predict a recent or an ongoing pollution from a given source and also predict a potential waterborne disease outbreak. In this study, the physicochemical parameters of coffee, tea and sugar effluent were determined before and after algal inoculation.

Phycoremediation studies for correcting the chemical nature of waste water from a conventional tannery treatment plant done in Europe Italy showed that the chemical and biological oxygen demands (COD and BOD) can be used to show an existing source of pollution or a recent introduction of an agricultural, domestic or even industrial pollutant into the waterbody.When determined the residual COD and BOD values were found to be compatible with the controlled discharge allowable limit values, with the study concluding that the contaminants were mainly from nonylphenol pollutants (Gregorio *et al.,* 2014).

The effect of sugar industry effluents on nitrate and phosphate levels in Rapti river,India established a significant elevation of nitrate and phosphate levels which resulted in increased eutrophication but decreased Dissolved Oxygen levels (DO) and hence lesser population of planktons (Nagendra and Nwaedozie, 2011). The results on investigation of the effects of sugar waste water on physicochemical parameters of soils at Haridwar area in India indicated significant increase in nitrate and phosphate levels in soil, consequently modifying the soil properties and quality. Hence soils irrigated with sugar effluents were likely to have increased nitrate and phosphate contents, increased pH but decreased moisture content. The high levels were due to the effect of mixing of effluents with the ground water around the sugar mill and also the contribution of animal wastes (Nagendra and Nwaedozie, 2011). Consequently these values were beyond those of the Indian permissible standards hence the water downstream was not considered suitable for drinking and irrigation purposes (Vinod and Chopra, 2010). Biochemical potential of brewery wastes co-digested with glycerol contained sugars which generated high levels of methane. Its co-digestion with glycerol enhanced biodegradation of brewery wastes hence had the potential to increase further production of methane (Deshmukh, 2014). Anaerobic treatment of waste waters with bacteria has the potential to generate methane (Costa *et al.,* 2013). Further Investigations on treatment of dairy and brewery waste waters with Bacillus and hydrolytic enzymes produced about 79% methane (Demirel *et al*., 2010). The waste co-digestion with glyceral has also been applied during treatment of animal dung to generate biogas (Costa *et al.,* 2013).

Wet processing of coffee is known to produce high quality coffee over the dry processing method. During wet processing, the exocarp and coffee pulp is removed via fermentation, a process that generates ethanol. In presence of methanogenic bacteria, ethanol is broken into ethanoic acid and eventually to methane and Carbon (IV) Oxide.The overall effluent also contains high levels of nitrate and phosphates initially present as nutrient fertilizers (Enden and Calvert, 2002). Whereas the above studies were known to release methane and carbon dioxide gases which are all environmental pollutants the current study aims at reducing the pollutants through the adoption of an algal based waste water treatment method which sequesters most of the methane gas and utilizes most of the nitrate and phosphates in the waste water hence reducing its public health impacts.

Phycoremediation studies by Dominic *et al.,*(2009) using *Synechocystis salina* were successful in treatment of waste water samples where the algal species reduced the phosphate

content of the waste water samples by 64.52%.The concentration of the phosphate declined substantially whereas the nitrate content decline rate was noted to be the highest and this stood at 96.23%. The waste water pH was also reported to have increased when it was treated with the *Synechocystis salina* (Dominic *et al.*,2009). Kotteswari *et al.,* (2007) noted in their study an increase in the waste water pH from 5.62 to 9.82 while Manoharan and Subrahmanian (1992), also agreed with the above findings of a progressive increase in the pH of the waste water inoculated with the algal species. Aarti *et al*., (2008) during an earlier phycoremediation study on waste water noted that the carbon dioxide gas exhaled by some aquatic plants and animals coupled with the bicarbonate chemicals presence in the waste water was the main cause of the decreased pH levels. On the other hand the onset of the photosythesis process meant that the available carbon dioxide in the waste water was to be utilized by the algae and therefore a decline of the carbon dioxide molecules lead to an increase in the pH level of the waste water. Consequently the decreased carbon dioxide meant that the oxygen molecules content in the waste water increased substantially (Aarti *et al*., (2008).

Chlorella vulgaris inoculated into waste water from different sources and held in various stabilization ponds had a substantial phycoremediation effect after 12 days incubation where the total nitrate contents of the waste water was highly reduced to appreciable levels (Pavasant *et al.,* 2006).The above findings highly correlated with those of Sreesai and Pakpain (2007), with the later reporting a shorter period of phycoremediation of 8 days.The shortened phycoremediation time was attributed to the natural sunlight incubation conditions mainly the light intensity of wavelengths 300nm – 700nm subjected to the waste water with 8 days net Phycoremediation effect whereas the waste water with 12 days Phycoremediation net effect had been incubated in artificial light (fluorescent light) which gave different light intensities (Yoshida *et al*.,2006).

Gonzalez *et al.*,(1997) while studying on industrial waste water found that *C.vulgaris* was able to remove 50% of the total phosphate from the waste water. Whereas according to a study by Weerawattanaphong (1998) on poutry waste water incubated with the *Chlorella vulgaris* for a period of 8 days noted a net phycoremediation effect of 77% total phosphate removal.Other studies on nutrient recycling using *Chlorella vulgaris* by Sreesai and Pakpain (2007) from the Bangkok city, Thailand reported almost similar total phosphate removal results and a substantial decline of COD from an initial of 90mg/l to a low of 20mg/l on the 8^{th} day of phycoremediation.

Mamun *et al., (*2012), in a study on industrial waste water treatment in Malaysia concluded that the logarithmic/progressive growth of *Chlorella vulgaris* was responsible for the total phosphate and total nitrate removal from the waste water.The growth peak of the *C.vulgaris* under natural light conditions (wave length 300-700nm) was on the 8th day of the batch culture beyond which little or no phycoremediation process took place and therefore the waste water was considered safe for discharge according to the standards of Malaysia.

A study by Kumar and Saramma (2012) on total nitrate and phosphate absorption by stationary algal cells of *Gloeocapsa gelatinosa* found out that the algae had an efficient phycoremediation effect on total nitrate and phosphates found in a waste water medium. The algal species absorbed 90% of the nitrate from the waste water medium within one day of incubation, whereas waste water medium with freed algal cells of *G. Gelatinosa* reported a less significant phycoremediation effect.The study showed that immobilized cells could be used for effective nutrient removal from a closed culture system.In other elaborate studies by Kotteswari *et al.,*(2007);Tam and Wong (1990); Chan *et al.,*(1979) and Anandaraj *et al.,*(2001) on phycoremediation of waste water, the efficacy and success of the algal species in phycoremediation of total phosphate was reported on day 10 and day 15 where 88 % and 90% of the total phosphate was removed under the laboratory conditions of artificial light.

More phycoremediation studies of waste water with an aim to reduce pollution load has also indicated pH of the pre-treated waste water as being 8.1 in day one and no change was found on the 5^{th} day but from 10^{th} day onward the pH decreased and by the 25^{th} day it had remained at about 7.1 (Sengar *et al.,* 2011).The reduction of the pH values following the phycoremediation of the waste water samples was also reported by Aarti *et al.,*(2008) using *C. vulgaris* and *C. salina* which showed a marked reduction in pH of the waste water treated with the respective algal species. The recorded pH values on subsequent phycoremediation tended to stabilize at 7.0 in water samples after the treatment with algae, where the algal treatment retained the pH of 7.0. Another related study on the application of phycoremediation technology in waste water by Rao *et al.,* (2011) an experimental research on waste water from a leather industry using *C.vulgaris* reported that on the 7^{th} day pH of the waste water was elevated from alow of 7.6 to a high of 8.0 and stabilized at that level thereafter.

Further studies on phycoremediation of dairy effluent using *Leptolyngbya* sp by Khemka and Saraf (2015) found that during treatment of the waste water using the algal isolate, pH of the medium increased constantly with algal growth up to 9.3 on 15^{th} day then decreased to 8.5 on 18th day and remained constant thereafter. Electrical conductivity showed irregular behaviours during phycoremediation. The reduction in total nitrogen content of dairy effluent, when inoculated with *Leptolyngbya species* having initial concentration of (2 x102 cells/ml) was observed at 60% level.

The determination of phycoremediation potential and the subsequent harvesting of energy from dairy industrial waste water through the anaerobic digestion by Kothari *et al*., (2012) clearly showed an increase in pH from neutral to alkaline and this was attributed to the increase in photosynthesis, enhanced algal growth and decrease in carbonates and bicarbonate levels of effluent medium. Rao *et al*., (2011) also showed that macroalgae *C. vulgaris* had a 90% nutrient removal efficacy during the phycoremediation process, and that the pH levels went up from 7.0 to 9.5 initially but later maintained stabilized at a value of 8.0. The photosynthetic process of the microalgae reduced the dissolved carbondioxide and bicarbonate concentrations and consequently raised the pH level of the waste water medium. Borowitzka (1998) conqured with the above findings and suggested that the bicarbonates required the carbonic anhydrase enzyme to convert them to CO_2. The pH levels had to be maintained so that the ammonia concentrations and the pH levels of the effluent did not get elevated as depicted in the equilibrium relationship below.

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$$

An Increase in the pH level presumably above 9.0 was thought to shift the reaction equilibrium to the left and thereby raising the concentration of ammonia. Hence, during phycoremediation the stabilization of the pH keeps the ammonia levels in check besides making the waste water pH levels conform to the set local and international discharge standards (pH 6-9).The above studies did not however compare the phycoremediation potentials and efficacies of different effluents and different algal species an aspect which the current study explored (Olguín, 2012).

Kshirsagar (2014) observed that phycoremediation of waste water resulted in a significant reduction of TDS values. The TDS reduction percentages using *C. vulgaris* were 68.42, 38.52, 43.37 33.47 %; and37.59, 34.40, 42.17,24.86 % in waste water treated by *C. salina*

respectively under laboratory conditions. The utilization of nutrients in the waste water mainly for growth by the algae was responsible for the reduction in the TDS levels (Rao *et al.,* 2011; Ahmad *et al.,* 2013).

Another comparative study on *Oscillotaria* and *Nostoc* which are blue green algae species on phycoremediation of waste water, Azarpira *et al.,*(2014) reported a success on TDS removal at the end of the incubation period. The combination of two algal species in the phycoremediation of waste water at low concentrations showed the best and most efficient TDS removal of 98 %. Similar results were reported by Kotteswari *et al*., (2007), Ahmad *et al*., (2013) and Elumalaei *et al*., (2013) who showed similar waste water phycoremediation results using various species of *Cyanophyceae* and *Chlorophyceae* with a mean success in TDS phycoremediation of up to 60 %. The bioabsorption process of the solid substances dissolved in the waste water by the algae was the main reason for the decreased TDS levels (Nanda *et al*., 2010). Rao *et al*.., (2011) studied on the potential use of phycoremediation process using waste water from a leather factory and his results highly correlated with those of Nanda *et al*., (2010) where the algal species under investigation immediately reduced the amount of total dissolved solids to about 1.3%. The consumption of the dissolved nutrients in the waste water by the *C. vulgaris* during the phycoremediation process and the subsequent conversion of the suspended solids into dissolved substances were responsible for the reduction in TDS levels.

Khemka and Saraf (2015) agreed with the above previous studies and reported that electrical conductivity of dairy waste water medium highly depended on the availability of ions, their concentration in the medium and their mobility and valency. The study showed that phycoremediation reduces EC value (dSm-1) from lag phase of the phycoremediation process to the stationary phase (18th day) and the absorption of the nutrients in the waste water medium and the subsequent algal growth was the reason behind the reduced electrical conductivity (Yu *et al*., 2005). The results were comparable with the decrease of EC from 0.55 – 1.45 dSm-1 to 0.27 – 1.36 dSm-1 (Mostafa *et al.,* 2012).

The COD and BOD tests depicts the toxicity and the oxygen requirement by algal species in breaking down the organic matter contained in a waste water sample (Ganapathy *et al.,* 2011; Abdel *et al.,* 2012). Phycoremediation of waste water using *S.salina* and *C.vulgaris* established that *S.salina* had better phycoremediation efficancy than *C.vulgaris* in the reduction of physicochemical parameters.The BOD values after phycoremediation using the

two algal species ranged from 83.17 to 90.63% and from 87.01% to 90.75% respectively, while the COD reduction efficiency ranged from 83.56 to 90.83% and 87.32 to 90.97% when treated with the two algal species respectively (Sharma and Shakeel 2013).The study therefore showed that the increased photosynthesis process and the high algal growth after the algal inoculations and the oxidation of the chemical pollutants in the waste water was responsible for the progressive reduction of both BOD and COD values, additionally the other reason might have been the conversion of the waste water organic matter to carbondioxide and the accelerated biodegradation process (Elumalai *et al*., 2013; Jayangouder *et al.*,1983; Abdel *et al.*,2012). Other phycoremediation studies on waste water by Sengar *et al*., 2011; Kshirsagar 2013; Azarpira *et al.*,2014; Khemka and saraf 2015;all agreed with the above study findings.

A pilot study by Wells *et al.,* (2013) on the effect of phycoremediation of a domestic effluent in South Africa reported a success rate of 87% bioremediation observed in the treatment ponds while the residual COD was contained in the algal biomass which remained behind. After phycoremediation the effluent released from the algal ponds was later proposed for farm irrigation thereby providing a mitigation measure for the water stressed areas of South Africa.In addition the study also showed that effective of algal biomass production could lead to self-shading as a result of diminished light intensity.

In Nigeria the effluent released from a paint industry company in Ebony was found to finally get into the nearby human settlement and this exposed the inhabitants to serious human health effects of pollution. In the study, the physico-chemical profiles and phyto-remediation of the paint waste waterwith N-Hexane de-fatted Moringa seed powder were studied by Otuu *et al.*,(2014). From the findings it was noted that the effluent was heavily polluted in terms of organo-eleptic properties of odor and color as well as high turbidity (18.20), conductivity (950 us/cm3) Total solids (1410.0mg/l) and Acidity (120.0mg/l). But after treatment with Moringa seed powder significant improvement was observed in the organoeleptic properties as well as turbidity (1.2), Total Solid (410mg/l), Acidity (23.0mg/l). The pH also improved from 8.0 to 7.50 after treatment, the researcher therefore noted that N-hexane de-fatted seed powder, had phyto-remediation potential in Ebony Paint effluent (Otuu *et al.,* 2014).

Raburu and Okeyo (2000) investigated the effect of agricultural industries on the water quality around Lake Victoria basin specifically on river Nyando in Kisumu County, Kenya and found substantial pollution by nitrates, silicates, ammonia and phosphates among other

pollutants. They attributed these pollution effects to agro-industrial activities, use of nitrogenous fertilizers for maize farming in the area as well as surface run offs. Empirical investigations on river Nzoia, pollution by Mumias Sugar Company in Kenya were done by Akali *et al*., (2011). From their findings, temperature and pH values did not vary significantly from site to site in the river, however, BOD, TDS and COD values increased significantly above the NEMA and WHO thresholds, hence necessitating the need to adopt methods that are highly efficient at pollutants removal. These two studies only highlighted the presence of the pollutants in the waste water but did not provide the remedial measures required in order to prevent public health effects and the environmental pollution once these contaminated effluent were discharged into the water bodies.

The study also did not address the problems associated with high levels of COD, BOD, nitrates and phosphates pollutants which pose a public health environmental challenge. The current study therefore endeavoured to reduce the high BOD,COD, nitrate and phosphate levels and assess them aganist the acceptable WHO values by providing a method highly effective in pollutant removal as recommended by Akali *et al*., (2011).

2.1.1 Comparison of the phycoremediation efficacies of *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa* in coffee, tea and sugar effluents

Different macroalgae are known to work effectively under different ecological set ups hence some of the macroalgae are well suited in highly polluted waste waters while others are well adapted to freshwater environments. Phycoremediation efficacies of these microalgae are therefore thought to be different when subjected to different ecological environments (Benemann et *al*., 2002).

In a phycoremediation study done by Sivasubramanian *et al*., (2012) on nutrient removal from coffee industry effluent in India, the selected microalgae namely, *Chlorococcum* sp, *Chlorella conglomerata* and *Desmococcus* spp were all separately inoculated into coffee effluent and incubated under laboratory and natural sunlight conditions.Upon determination of the phycoremediation potential all the three species of algae were noted to have reduced the nutrient pollutants contained in the effluent substantially. However variation in the nutrient removal efficacy was noted in the effluent incubated under laboratory conditions and those under the natural light conditions, whereby those incubated in the natural light conditions had a higher phycoremediation potential than those incubated in the

laboratory.The difference in light intensities experienced in the two incubation conditions were responsible for the phycoremediation variations (Benemann *et al*., 2002).

Microalgae are ubiquitous set of microorganisms and this is because of their ability to absorb the nitrates and phosphate nutrients differently depending on their availability levels in the waste water (Ayodhya., 2013).The phycoremediation variability is usually on the ratio of 1:10 for the nitrates and phosphates nutrients respectively (Benemann *et al*., 2002). Besides that, microalgae cultures possess the ability to fix carbon dioxide leading to the release of biofuels providing a significant solution for the greenhouse gas emission (Benemann *et al*., 2002). Ayodhya (2013) conducted a related study on comparison of pollutant removal efficiencies of algae. In the study the phycoremediation of nitrate pollutants using *Chlorella vulgaris* and *Scenedesmus quadricauda* species of algae from a polluted effluent was estimated. Upon phycoremediation it was found that the removal of nitrate from the waste water was significant with *C. vulgaris* and *S. quadricauda* up to 15th day. *C. vulgaris* was therefore found to perform better than *S. Quadricauda* in the nitrate removal and this clearly demonstrated the effectiveness of algae in the absorption of nitrates and other waste water nutrients in general and that *C.vulgaris* was the best algal species for use in phycoremediation studies.

Muhammad and Nwaedozie (2011) through their research on sea weed affirmed that the weeds had the carboxyl and sulphate groups on the outer surface of the cells, and these groups are the ones thought to be responsible for the binding of nitrates and phosphates by the seaweed.Algae like the brown algae species have high very high amounts of carboxyl groups which can aid in bioabsorption of the nitrates and phosphates from the waste waters.The pH of the waste water medium always plays a critical part inthe functionalities of these groups thus inducing an effective adsorption and eventual absorption of the nutrients (Benemann *et al*., 2002).

*Chlorella*is a genus that belongs to phylum *Chlorophyta.*The algae contains both chlorophyll-a and –b green photosynthetic pigments.The most important species of this genus is the *Chlorella vulgaris* which apart from being used as a food supplement it is also highly used in phycoremediation studies and this is due to the fact that this algae has a lot of functional groups on its cell wall (Lesmanaa *et al*., 2009). Thereby the species is able to bind with different pollutants in the waste water thereby effectively removing or reducing them to appreciable levels. The algal species cell wall is also porous one allowing the free passage of

nitrates, phosphates and other pollutants from waste water into the inside of the cell. These two biabsorption properties makes the *Chlorella vulgaris* the most suitable algal species for phycoremediation (Lesmanaa *et al*., 2009).

Murali and Nisha (2009) compared the relative phycoremediation abilities of *Chlorella vulgaris* and *Gloeocapsa gelatinosa* to absorp and reduce the pollutant load namely nitrates and phosphates of polluted waste water samples collected from industrially polluted regions. The physicochemical parameters measured before and after phycoremediation process showed the effectiveness of the three algal species in waste water treatment. The study showed that after phycoremediation the polluting parameters were reduced to much lower and acceptable levels and that during the incubation period some parameters like pH increased instead of reducing and this was directly related with the rate of photosynthesis whereby the carbon dioxide and the waste water bicarbonates were utilized by the algae thus increasing the pH .The increased photosynthesis lead to increase of algal biomass and if this was not checked upon death and decomposition of the algal plants the situation would reverse back whereby the carbon dioxide would increase reducing the pH of the medium.*Chlorella vulgaris* absorbed more pollutants than and *Gloeocapsa gelatinosa* which was the least performing methane and nutrient biosorbent algal species. BOD,COD,TDS and EC of the waste water were better phycoremediated using *Chlorella vulgaris*. Murali and Nisha (2009) therefore concluded that *C.vulgaris* spp were more efficient in uptake of nitrates relative to and *G.gelatinosa* spp. The decreasing order of nitrate removal efficiency was *Chlorella vulgaris*>*Gloeocapsa gelatinosa* (Cyanophyceae). The study however did not subject the above various algal species to the different waste water so as to show the differences in pollution tolerance among these algal species a research gap which this study aims at addressing.

Similar phycoremediation studies were done by Azarpira *et al.,* (2014) using mixed *Cyanobacteria.* In their study, efficiency removal of nitrates, phosphates and sulphates were investigated using *Cyanobacteria* species namely *Oscillatoria limosa* and *Nostoc commune*. Nitrate removal by the two algae was very efficient with phycoremediation efficiency success of 97% and 96% respectively. The same order was plausible for phosphorus with *Oscillatoria limosa* (93%) and *Nostoc commune* (84%). Similarly for sulphate, *Oscillatoria limosa* outweighed *Nostoc commune* with removal efficiencies of 95.8% and 92%, respectively. From the study it was noted that algae were efficient in the removal of the nutrient pollutants,

however the algal species needed to be subjected to various contaminated waste water so as to show the pollution tolerance and nutrient removal efficiencies among the algal species.

Sahu (2014), investigated removal of organic and inorganic pollutants of waste waters using *Chlorella vulgaris* in Ethiopia. The maximum reduction of COD and BOD were observed after 21 days, nonetheless, maximum reduction in the level of phosphates and nitrates were observed in 15 days. From the findings 70% BOD, 66% COD, 71% total nitrogen and 67% total phosphorus were removed by the selected Algae, implying that *C. vulgaris* is efficient at removal of nutrients from waste waters. Related studies conducted by Saranraj and Stella (2012) on phycoremediation of sugar effluents using a mixture of stationary bacterial cells (5% Inoculums) showed that the sugar effluents had high levels of phosphorus, nitrogen, TSS, BOD and COD, however, treatment of the effluents with the bacterial consortium containing *Bacillus subtilis, Serratia marcescens and Enterobacter asburiae* under aerated conditions significantly reduced the pollutant levels after 6 months. From the study the researchers deciphered the advantages of this method as being not only eco-friendly, but also economical, efficient and easy to use. However from the earlier studies by Sahu (2014) the algal species were noted to remove the pollutants within a studied period of 15 days. The above study on bacterial species had along pollutant removal period of 6 months and did not consider the efficacy of the algae on reducing the physicochemical pollutants notably COD, BOD and the environmental impacts of the effluent to the studied population, an aspect which the current study researched on through phycoremediation of the effluent.

Kurt and Jessica (1995) studied on phycoremediation efficiency on three selected macroalgae in Unguja Island, Zanzibar, Tanzania and concluded that local macroalgae were suitable for efficient nutrient removal and suggested that a system with simple tanks or ponds with macroalgae can be used for small-scale phycoremediation of waste water in the region. Raburu and Okeyo (2000) on waste water discharge from agricultural activities, Kenya conqured with the above studies and suggested that a treatment process with high efficiency and low energy input was needed thus strongly advocating for macroalgae treatment (phycoremediation) of domestic, industrial and agricultural waste water. The current research therefore endeavoured to fill the phycoremediation study gaps left by the previous researchers through demonstration of the phycoremediation efficacy in the treatment of the various agricultural waste water.

2.2. Phycoremediation efficacies of *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa* on nitrates and phosphates in coffee, tea and sugar effluents aganist WHO permissible stds.

Assessment of the phycoremediation efficacies of the three algal species was done by estimation of the nitrates and phosphates levels in tea, coffee and sugar effluents before and after the specific algal inoculation against the WHO standards.

Contaminated effluent is always subjected to a sequential flow during the treatment process inorder to reduce eutrophication brought about by nitrates and phosphates (Larsdotter, 2006). Primary treatment of waste water aims at removing or scopping away the visibly large materials in the grid system of the treatment plant brought along with the waste water. Whereas the increased aeration in the secondary pond increases the amount of the dissolved oxygen and sets the stage for the aquatic habitation of the would have been waste water into useful water (Larsdotter, 2006).In this stage waste water is pumped into a tank packed with plastic blocks by way of spraying the waste water through a sprinkler system as the water percolates down the tank then into the secondary ponds a biofilm of organic substances is always left on the plastic blocks which is normaly acted upon by the aerobic bacteria releasing carbon dioxide whereas the bacteria are normally fed on by protozoans. The dissolved carbon dioxide is inturn utilized by the photosynthetic algae in the secondary and tertiary ponds which inturn releases oxygen molecules into the waste water thereby reducing the biochemical oxygen demand thus increasing the oxygen content of the waste water on its discharge (Larsdotter, 2006).

Nitrates and phosphate pollutants found contaminating waste waters are mainly removed by way of absorption and assimilation into the algal cells and if the amounts of these nutrients are high in waste water then the effects of eutrophication may be experienced in the waste water flow channels and in the receiving water bodies. Eutrophication may lead to increased BOD and other physicochemical parameters which may need assessment against the WHO standards if the initial river integrity is to be restored (Larsdotter, 2006; Veele, 2012).

Phytoremediation evaluation of the nutrient pollutant removal efficiency by three plant species conducted in Poland by Marecik *et al.*,(2013) established that the uptake and removal rate of the three plant species was highly dependant on the infiltrability of the plants by the nutrient pollutants coupled with the contact period of the waste water containing the nutrient pollutants and the individual plant species.For the first nine days the nutrient uptake by the

plants was high with the up take amount varying among the plant species and then in the following subsequent days a low nutrient uptake was noted with the stationary uptake period noted at day 21 of the study period. The phycoremediation efficacy was 82% and 79% for the sweet flag and common reed plants respectively.

Another study done to evaluate the efficiency of yeasts *Candida zeylanoides and Saccharomyces cerevisiae* pure isolates from soil for the textile bioremediation of waste water incubated for 15 days in a flask showed that *S.cerevisiae* was the most effective microorganism in the waste water treatment with a mean of 66% reduction in nitrate and phosphate levels *C. zeylanoides* had a 57.3% success reduction in nitrates and phosphates, but on mixing the *Candida zeylanoides and Saccharomyces cerevisiae* a much lower nutrient remediation efficacy of 50% was noted (Abioye *et al.,* 2014).

Rajasri and Goutham (2013) examined the reduction of nitrates and phosphates from a dairy effluent and reported significant decrease in nitrogen and phosphorous levels due to algal uptake. Phosphates were removed with an efficiency of up to 97%, whereas nitrates were removed at an efficiency of 96%. Kshirsagar (2014) noted phycoremediation of effluents is always followed by enhanced declines in the nutrient content of the effluent samples. *Chlorella vulgaris* and *Chlorella salina* were all effective in the nutrient removal contained in the effluent mixtures and that subjected to the same conditions and inoculation concentrations *C.vulgaris* was observed to have a better phycoremediation success than the *C.salina.* However when calibrated with an algae free effluent the algae inoculated effluent were shown to provide an inside knowledge on the usefulness of the phycoremediation process. Similar results were also recorded in another study by El-Sheekh *et al.,* (2015).

Khemka and Saraf (2015) studied on the phycoremediation of dairy waste water coupled with biomass production using *Leptolyngbya* sp. From the study, nitrate concentration was reduced by 43.80% on 9^{th} day then concentration further increased up to15^{th} day. Nitrite concentration was reduced by 61.3% on 18th day. Total phosphorus was reduced tremendously by 52.3% with 87.4 mg/l concentration remaining in the media on 18^{th} day of remediation experiment. Also, total phosphate showed a significant removal difference ($p < 0.05$) especially when compared with the algal biomass production. Orthophosphate concentration decreased up to ~50 % on 18^{th} day, with the increased pH and biomass. Azarpira *et al.,* (2014) studied on the effectiveness and the potential use of *Oscillotaria* and *Nostoc* in the treatment of Municipal waste water and the final phycoremediation of the

physicochemical pollutant results indicated that the two species had almost the same phycoremediation efficacy. The results compared very well with the findings of Kshirsagar, (2013) who reported a similar nitrate phycoremediation success using *Scenedesmus* and *Chlorella* species.

Rao *et al.*,(2011) noted that phosphorous nutrients are highly required for the growth of micro-algae *C. vulgaris* and that its consumption from a waste water sample will automatically lead to a reduction of the same nutrient.Once actively absorbed and assimilated in the algal cells the phosphorous has various functions besides the production of the algal biomass (Becker, 1994).The study further established that the phycoremediation efficiency and success of *C. vulgaris* was 100% in the waste water and this was due to the high photosynthetic process in algal inoculated medium (Hammouda *et al.*, 1994). Moreover, the micro-macroalgae absorbs the phosphorus nutrients in large amounts, which are then kept inside the algal cells in the form of polyphosphate granules (Bitton 1990).

Rao *et al.*, (2011) noted that excess nitrates had serious environmental and public health effects like methemoglobin a blood disorder caused by excessive nitrates in water. The presence of the elevated levels of nitrogenous compound in water is a known environmental pollutant which can lead to a high BOD level thus affecting the integrity of the river receiving such pollutants because of the increased demand for the dissolved oxygen. Nitrite is awidely known environmental pollutant of a greaterpublic health concern because its one of the major chemical pollutant and nutrient limiting substance in agricultural waste water (Sedlak 1991).Nitrites can lead to methaemoglobin formation, a compound which has a high affinity of oxygen molecules and highly displaces the normal haemoglobin. Interestingly, Oliver and Ganf, (2000) reported that all forms of nitrogen are absorbed by algae.Khazenzi *et al.*,(2013) in astudy done in Eldoret Kenya, showed that ground water and surface water is likely to be contaminated by fertilizers from agricultural areas during rainy season and therefore present public health concerns like infantile methaemoglobin especially when the water is used for domestic purposes.

Rao *et al.* (2011) in his study on waste water reported that *C. vulgaris* were able to phycoremediate against the different forms of nitrogen pollutants drastically reducing their levels to the acceptable WHO standards of 10mg/l. He also noted that NH_4 could be lost into the atmosphere especially when the pH is elevated as it happens in enclosed algal cultures (Nunez *et al.*, 2001).

Phycoremediation studies on tannery effluent using a marine algae *Chlorella salina* on nutrient removal using immobilized cells and free cells of the macroalgae, Jaysudha and Sampathkumar (2013) found out that the immobilized cells of the macroalgae were able to take up phosphates from tannery effluent continuously within their exponential phase of the cultures. From the study, it was found out that the immobilized *C. salina* showed a very high efficiency potential in phosphate removal of 99.39 (8.21mg/l) as compared to the free cells which showed a removal efficacy of 81.94% (6.76mg/l) from tannery effluent on the 8th day of culture. Kumarand Saramma (2012) in their study on phycoremediation also reported that 80% of phosphates were absorbed by immobilized cells of macroalgae (*Gloeocapsa gelatinosa)* cultured in harvested effluent. Similarly, the chitoson immobilized cells of *Scenedesmus* sp also showed a high efficient of about 90% in phosphate removal after a period of 9hrs and 94% after 12hrs respectively (Sashenka *et al*., 2008; Tam and Wong, 2000).

In a pilot study done in South Africa by Wells *et al.,* (2013) the algal species used showed an enhanced overall nutrient removal upon assessment, however the ponds without the algal inoculums did not have any nutrient pollutant variation. Phycoremediation studies on various seaweeds done in Unguja Island, Zanzibar, Tanzania, showed high uptake rates for ammonium, nitrate and phosphate in the three selected algae species. Nutrient load and composition, mainly N:P ratio, were varied to optimize uptake efficiency with the research concluding that local macroalgae were suitable for efficient nutrient removal (Kurt and Jessica 1995).

In Kenya results from the daily monitoring of effluents contaminated with nitrates and phosphates within the Eldoret Municipal treatment plants showed that the tertiary and the maturation ponds of the waste water treatment which normaly has the natural algae growing had a low nutrient content, BOD and COD as compared to the ponds without the algae (ELDOWAS 2015 Weekly reports).

From the above studies it was evident that algae are better in remediating industrial and agricultural effluents than bacterial and fungal organisms however most of these studies did not consider the phycoremediation efficacy of *C.vulgaris, S.salina* and *G.gelatinosa* on nitrates and phosphates in coffee,tea and sugar effluents against the WHO standards. The current study aimed at reducing the COD and BOD levels besides decreasing nitrates and phosphate content within different effluents through phycoremediation.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Design

This study adopted experimental study design. Oso and Onen *(*2009) elaborated that experimental study design involves a systematic manipulation of some characteristics and examination of the outcome. This design is mainly employed in testing cause-effect relationship through manipulation of variables.From the study the concentration values of the studied variables from the three effluents were all expected to reduce after treatment with either of the three algal species namely *C.vulgaris, S.salina* and *G.gelatinosa* thus phycoremediating on the pollutants likely to cause a myriad of waterborne diseases from the contaminated tea, coffee and sugar waste water.

3.2 Study Site

The study was carried out in selected waste water from coffee, tea and sugar factories in the respective sites namely Bungoma, Nandi and Kakamega. Bungoma,Nandi and Kakamega were used as the sampling frames from where representative samples were drawn from. These sampling frames were appropriate because Nandi had seventeen tea factories, Kakamega with three sugar factories whereas Bungoma had sixteen coffee factories. The criterion used in selection was the high presence of coffee, tea and sugar effluents within these counties, proximity of the counties to the research laboratory for easy of sampling and transportation and also the presences of streams and rivers into which these effluents are discharged into. Purposive random sampling was therefore used to select the factories to be included in the study. The selected counties therefore included Bungoma (coffee effluent), Nandi (Tea effluent) and Kakamega (sugar effluent). All the functional factories were identified from each selected county and serial numbers assigned.The factories were then selected randomly based on the serial numbers already given. Locations of selected factories were geo-referenced using GPS as indicated in plates 3.1- 3.5

Study Areas

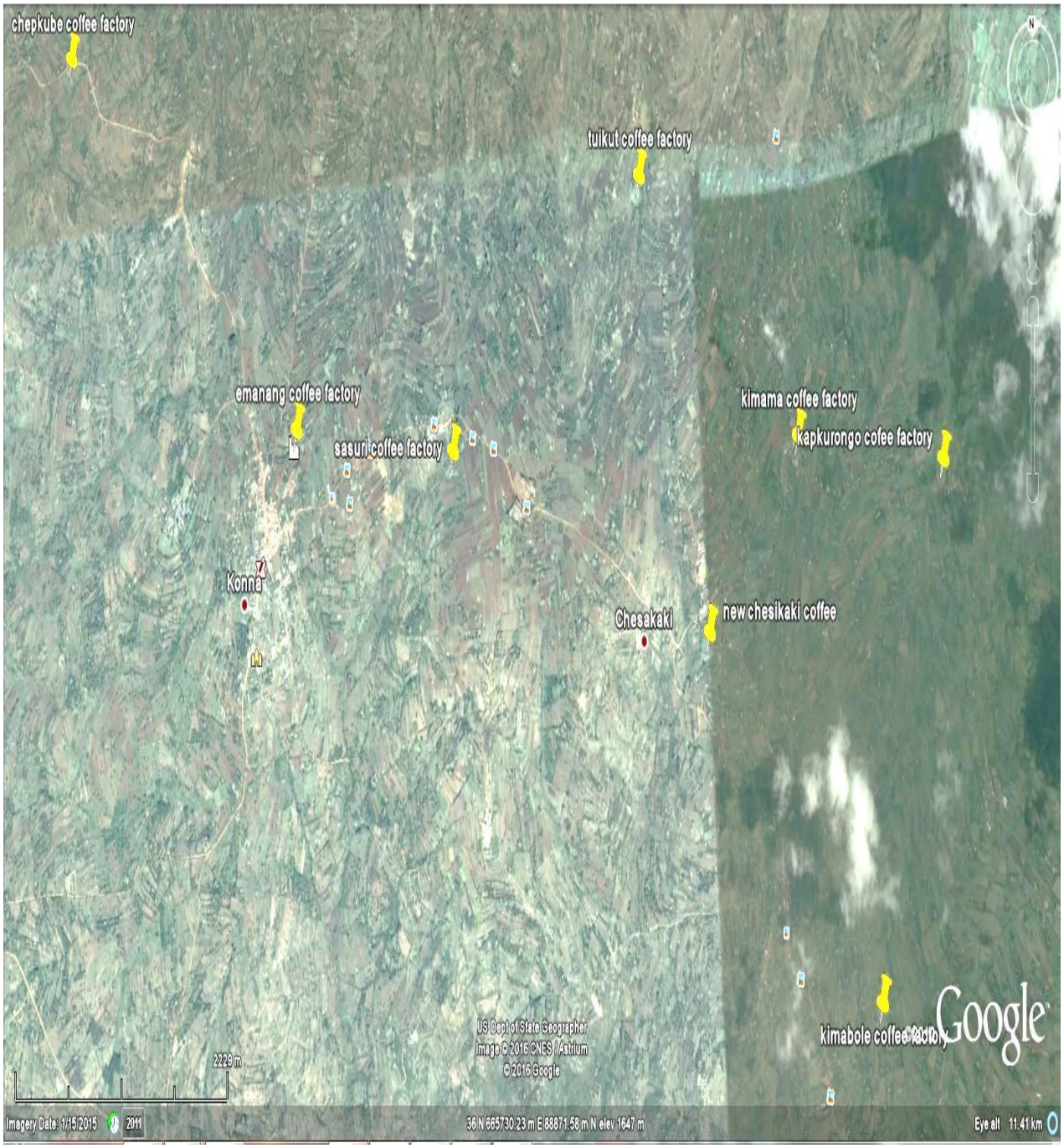


Plate 3.1: Bungoma County 0.84790N,34.70200E (Mt. Elgon sub county) coffee factories (courtesy of KPLC 2015)

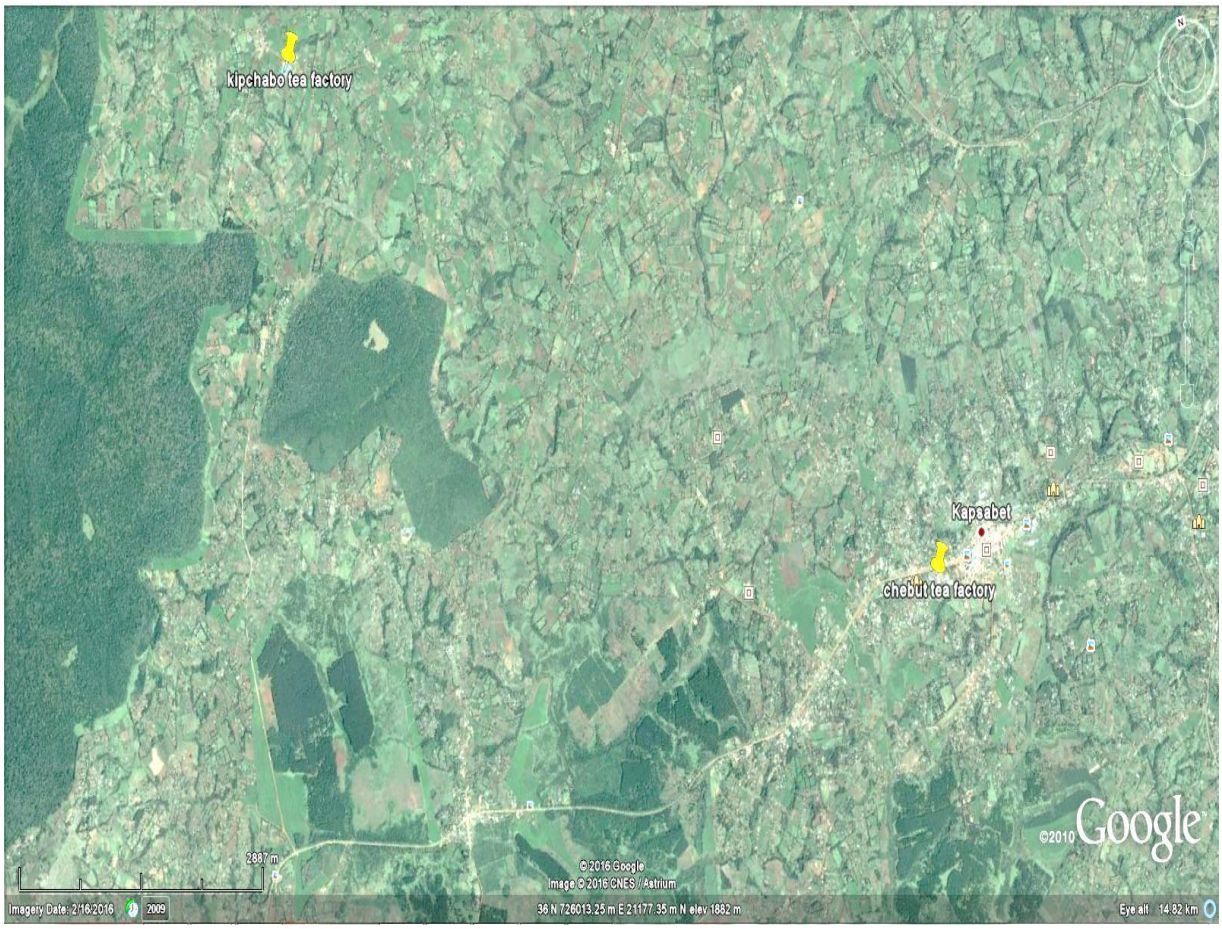


Plate 3.2: Nandi County 0.1036^{0}N, 35.1777^{0}E; Tea factories (courtesy of KPLC 2015).

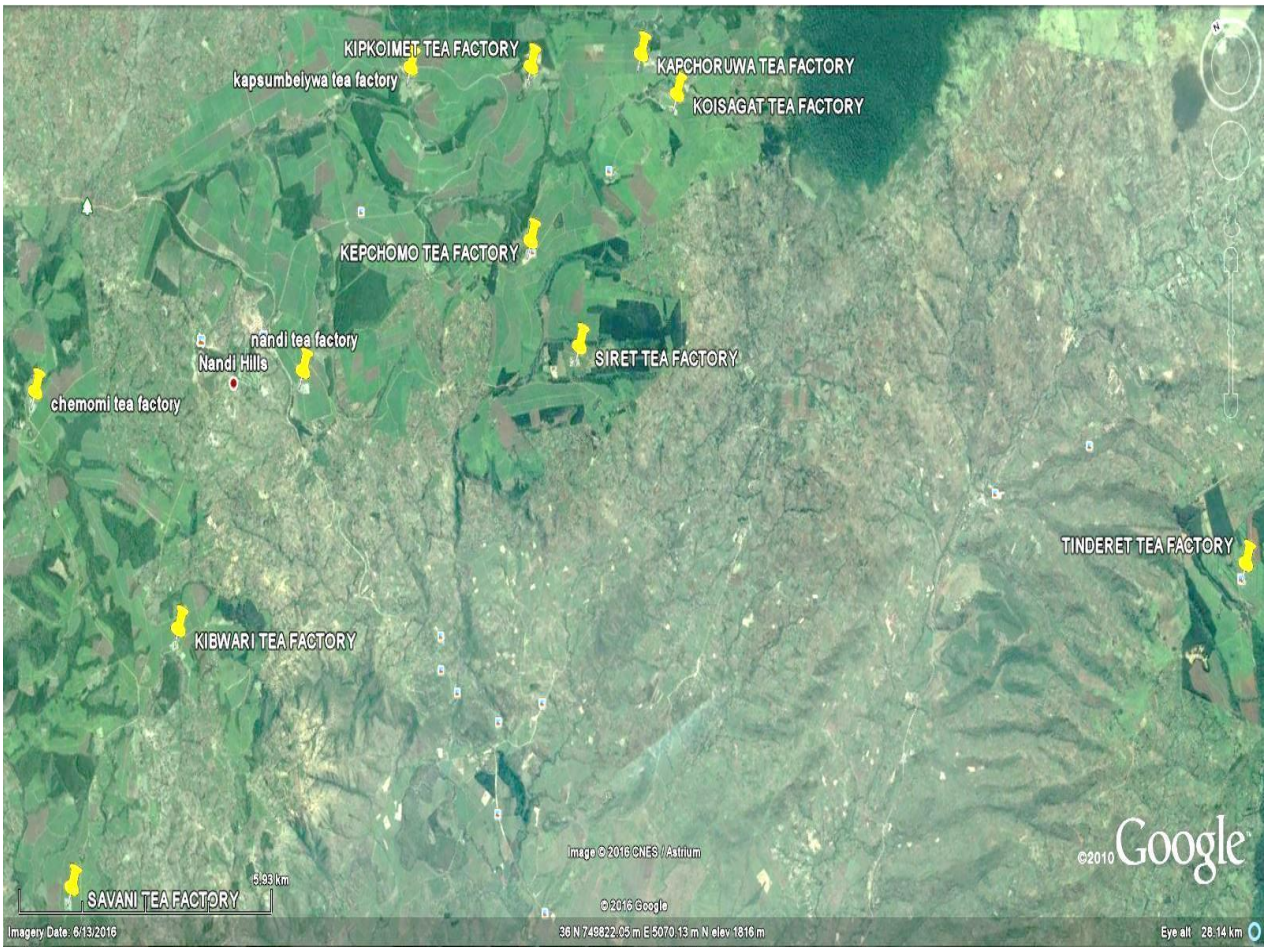


Plate 3.3: Nandi County 0.1036^{0}N,35.1777^{0}E; Tea factories (courtesy of KPLC 2015).

Plate 3.4: Kakamega County 0.2837^{0}N, 34.7515^{0} E; Western Sugar company in (courtesy of KPLC 2015).

Plate 3.5: Kakamega County 0.2837 ^{0}N, 34.7515 ^{0} E; Butali Sugar company in (courtesy of KPLC 2015).

3.3 Inclusion and Exclusion Criteria

3.3.1 Inclusion Criteria

All the functional factories which were operational at the time of the study were all considered for inclusion in the study and were all labelled alphabetically from A-Z for easy of random sampling**.**

3.3.2 Exclusion Criteria

All the non-functional factories and the ones which failed to consent were not considered thus they were eliminated from the study since they could not produce reliable data.

3.3.3 Sampling Method

Composite waste water samples which provided a better representation of the heterogeneous mixture of algal species were collected from waste water treatment ponds in Pan paper company Webuye mainly for the isolation and identification of *Chlorella vulgaris, Synechocystis salina and Gleocapsa gelatinosa* for use in the study of phycoremediation of effluents from the tea, coffee and sugar factories. The treatment plant had waste water treated in three stages mainly the primary, secondary and tertiary treatments then the final effluent discharged into the nearby river Nzoia. A sampling water jar was immersed at full depth at the primary pond and the waste water filled in a sterilized two litre plastic container (APHA 2005). The same procedure was used in sampling the subsequent secondary and tertiary ponds of the waste water. The sampled waste water was then secured safely in a potable cool box and transported immediately to the Eldoret water and sanitation company laboratories for identification and subsequent isolation of the algal species notably *Chlorella vulgaris ,Synechocystis salina* and *Gleocapsa gelatinosa* to be used in the study. When waste water was cultured the *Gleocapsa gelatinosa* colonies appeared cream whitish in colour while *Synechocystis salina* colonies appeared orange in colour while the *Chlorella vulgaris colonies* appeared green yellow in colour when grown in the nutrient agar as referenced from an handbook of microalgal culture by Richmond (2004). The algal species colonies were microscopically observed and identified as shown in the plates 3.11;3.12 and 3.13 (Richmond, 2004).

From the study areas sampling was done from the waste water flow channels from all the functional factories under study where the discharge points from the factories to the streams and rivers were noted. It was found that most of the factories did not have treatment ponds and therefore discharged their effluents directly to the nearby streams and rivers.The points of

contact of the waste water and the stream/river were therefore picked as the sampling points. Grab samples were taken at the middle of the effluent flow using a sealed container which was fully immersed into the water column then opened and filled before sealing again without a gap. Temperature, pH and conductivity measures were all taken on site. The samples were then stored in a cool box then transported to the Eldoret University and Eldoret water and sanitation company laboratories where the algal culture, BOD, COD and physicochemical parameters were all done (APHA 2005)

Purposive and random sampling were used whereby once all the functional and willing factories had been identified, the factories were then serialized alphabetically and the numbers all put in one bucket, mixed and then picked randomly for inclusion in the study.

3.3.4 Sample Size Determination

Because of the finite population of the factories to be sampled Yamane, (1991) method was used to calculate the sample size required. .

$$n = \frac{N}{1 + Ne^2}$$

Where n=sample size, N=population size, e=the error of sampling.

N= 17

e=0.05

Therefore n=17/1+17(0.05)^2

n=16

Therefore 16 tea factories were included in the study.

Eight functional coffee factories were found in Mt. Elgon of Bungoma County and the sample size was calculated as follows;

$$n = \frac{N}{1 + Ne^2}$$

8/1+8(0.05)^2

n=7.8

Therefore 8 coffee factories were also included in the study

$$n = \frac{N}{1 + Ne^2}$$

n= 3/1+3(0.05)^2

=2.9

Therefore 3 sugar factories were to be considered but only 2 sugar factories were included in the study because in one of the factories the permission was not granted. Sample size was

thus 26 factories i.e. 16 tea, 8 coffee and 2 sugar, hence after calculation only 26 factories were sampled.

3.4 Experimental Layout

In the laboratory identification of the algal species mainly *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa* for use in the study and other algal species which were not of study interest were done through wet preparation and microscopy techniques. 500mls of the nutrient agar medium were prepared from the stock ingredients and the pH carefully adjusted to 6.8-7.0 using 1 molar KOH with a magnetic stirrer and hot plate. The media was autoclaved at 121^{o}C for 15 minutes then allowed to cool for 10 minutes under room temperature before 20mls were dispensed in petri dishes. Using standard plating technique, aloop full inoculums of the waste water containing the mixed algal species were inoculated into the prepared plates of nutrient agar medium, this step was done aseptically according to (APHA 2005).

The algal inoculated plates were placed in cotton moisture tray and incubated at 25^{0}C room temperature and placed next to a glass window in the laboratory to provide the natural light (300nm-700nm) with fluorescent tube light being left on for 48 hours incubation period. Other plates were also incubated in an incubator with controlled temperature conditions of 25^{0}C for optimal growth. After 48 hrs of incubation the different algal species were identified through the various colony morphology and pigmentation. The different colonies were picked with a sterilized wire loop and inoculated into a fresh nutrient agar medium to allow growth of the axenic algae species only (APHA 2005). The plates were incubated under light conditions for 48 hrs after which specific algal isolates of *Chlorella vulgaris, Synechocystis salina and Gloeocapsa gelatinosa* were identified microscopically referring from an hand book on microalgal culture by Richmond (2004) as shown in plate 3.11, plate 3.12 and plate 3.13 then sub cultured for subsequent inoculations into the already sterilized respective waste waters. The axenic cultures were maintained at a thermostatically controlled refrigerator to prevent contamination and further growth and also maintain their viability for subsequent use (APHA 2005).

Serial dilutions of the axenic cultures of *Chlorella vulgaris* (Chlorophyceae), *Synechocystis salina* (Cyanophyceae), and *Gloeocapsa gelatinosa* were made by picking a standard loopfull colony of each species and mixed it with the first tube of 1:1 dilution factor and then transferred to the next tube containing 10mls physiological saline and the same repeated to

the subsequent 10 tubes and dispensing off 1ml in the final tube after mixing the contents. Using the standard neuber chamber (heamocytometer) for estimation of cell numbers, algal cells in each tube were counted microscopically.

3.5 Determination of the phycoremediation efficacy of *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa* on the physicochemical parameters of coffee, tea and sugar effluents.

The initial physico-chemical analysis of the waste water samples were made before and after inoculation of the specific algae, the total content in each beaker was filtered to remove algae and then used for the analysis of various parameters (pH value, total dissolved salts (TDS), biological oxygen demand (BOD), chemical oxygen demand (COD), nitrate, and phosphate) (APHA 2005)

3.5.1 Preferentail Hydrogen Analysis (pH)

Electrometric method

The pH determination was done using the electrometric method, which was the most accurate and interference free method.

Principle

The pH values for all the samples were determined by measurement of the electromotive force (emf) of a cell comprising of an indicator electrode (an electrode responsive to hydrogen ions such as glass electrode) immersed in the test solution and a reference electrode (usually a calomel electrode). Contact was achieved by means of a liquid junction, which formed a part of the reference electrode. The emf of the cell was measured with pH meter. Since the pH is defined operationally on a potentiometric scale, the measuring instrument was also calibrated potentiometrically with an indicating (glass) electrode and a reference electrode using standard buffers having assigned pH value so that,

$pHB = -\log_{10} [H+]$

Where pHB = assigned pH of standard buffer.

The operational pH scale is used to measure sample pH and is defined as:

pHs = pHB + F (Es – EB) / 2.303 RT, where;

pHs = potentiometrically measured sample Ph

F = Faraday 9.649 x 104 coutomb/mole

Es = Sample emf V

EB = Buffer emf V

R = Gas constant 1.987 cal deg-1 mole-1

T = absolute temperature, °K.;The apparatus, equipments and the reagents for pH estimation are indicated in appendix I

3.5.1.1Calibration of the electrode

Before use, the electrodes were removed from the water and rinsed with distilled water. The electrodes were dried by gentle wiping with a soft tissue before they were calibrated.

Calculation

The pH value was obtained directly from the instrument (APHA 2005)

3.5.2 Conductivity Measurement

Principle

This method was used to measure the conductivity generated by various ions in the waste water. The dissolved ionic contents contained in the waste water sample was made by multiplying specific conductance (in mS/cm) by an empirical factor which may vary from 0.55 to 0.90 depending on the soluble components of the waste water and on the temperature of the measurement. The conductivity measurement gave a rapid and a practical estimate of the variations in the dissolved mineral contents of the waste water (APHA 2005)

Reagents and standards

The conductivity of the water was made to be less than 1 mmho/cm; Standard potassium chloride of 0.01M was made by dissolving 745.6mg anhydrous KCl in conductivity water and topped up to1,000ml at 25°C. This was the standard reference solution, which at 25°C had a specific conductance of 1,413mmhos/cm. The solutions were stored in glass stoppered Pyrex bottles at 25^{o}C room temperature.

Procedure

Conductivity was measured as per the instruction manual supplied with the instrument and the results expressed as mS/m or mS/cm. The temperature at which the measurements were made was also noted accurately. Conductivity meter needed very little maintenance and gave accurate results once calibrated (APHA 2005). Measurement of conductivity was done according to manufacturer's instructions as shown in appendix II

3.5.3 Biological Oxygen Demand (BOD_5)

Determination of BOD by trak machine incubator

Procedure

The waste water sample was heated to 20^{o}C of its incubation temperature then using a clean and graduated glass cylinder, 100ml waste water sample volume was poured into a BOD trak sample bottle containing aclean magnetic stir bar of 3.8cm as shown in plate 3.6 which showed the set up of the BOD and COD.The BOD nutrient buffer pillow contents were introduced into each bottle containing the waste water inorder to facilitate optimum bacteria growth.The stopcock grease was applied to the seal lip of each bottle and to the top of each cup then a seal cup was placed in the neck of each well stoppered bottle.Lithium Hydroxide powder pillow was added to each seal cup using a funnel. The powder particles were not allowed to fall into the waste water sample and incase this occurred, the waste water sample was discarded and fresh one prepared (APHA 2005).

The prepared bottles were then placed on the chassis of the BOD Trak machine. The appropriate leading tubes to the sample bottles were then connected and firmly the caps were tightened accordingly. Each tube was tagged with the channel number of the bottle containing a specific waste water and the channel number setup was reflected on the common control panel.The BOD trak machine was then started (after connecting the electrical plug and turning the instrument on) after making sure that all the stir bars were rotating. If a stir bar slid to the side of the bottle, then the bottle was lifted off the unit and replaced to the channel until the stir bar was rotating properly before the channel number was reset again. Once the incubation period was over a graph was displayed on the machine screen.The BOD_5 results for each sample were read directly from the BOD **t**rak display by pressing the key corresponding to the each sample then the results of each sample test were recorded on the research book (APHA 2005).

The bottles were then removed from the trak and emptied in preparation for another sample incubation while a brush and hot soapy water was then used to clean all bottles, stir bars and seal cups after which the bottles were rinsed thoroughly with distilled water then dried.

3.5.4 Chemical Oxygen Demand (COD)

Procedure

Using the apparatus and reagents in appendix III a 0.4g mercuric sulphate ($HgSO_4$) was placed in 250ml refluxing flask then mixed and diluted with 20 ml distilled water. 10ml

standard potassium dichromate (0.25N) and glass beads previously heated to 600 degrees centigrade for 1 hr were then added into the mixture. The 250ml reflux flask was connected to the condenser and slowly 30ml conc H_2SO_4 containing silver sulphate was added through the open end of the condenser then mixed by swirling beforere fluxeingthe mixture for 2hrs as shown in the plate 3.6 of the COD reactor.The condenser was then carefully cooled and washed down with distilled water then the mixture was diluted to about 150ml with distilled water and cooled to about 20^{o}c.The excess dichromate was then titrated with ferrous ammonium sulphate using 0.1-0.2ml ferroin indicator to reddish brown end point then a blank was refluxed in the same manner (Andrew *et.al.,* 1995).

Calculation

COD (mg/l)= (a-b)Nx8000/ml of sample taken

a= blank titre

b= sample titre

N= normality of ferrous

s ammonium sulphate. (Andrew *et.al.,* 1995).

Plate 3.6: Waste water samples incubated in a COD reactor for two hours and the BOD trak bottles (photograph taken on 18.6.2015; by A. Mbeke).

3.5.5. Total Dissolved Solids (TDS)

Principle

Residue which were left after the evaporation and subsequent drying in oven at specific temperature 103-105°C of a known volume of sample were the total solids. Total solids included "Total suspended solids" (TSS) and "Total dissolved solids" (TDS). Whereas loss in weight on ignition of the same sample at 500°C, 50°C, in which organic matter is converted to CO_2 volatilisation of inorganic matter as much as consistent with complete oxidation of organic matter, are volatile solids.

Total dissolved solids

The filterable residue included all the materials that passed through the standard glass filter disk and remained after evaporation and drying at 180°C. The apparatus and the equipments used were described in appendix iv

Sample collection, preservation and storage

TDS analysis was done immediately after collection of the waste water sample due to impractically of preservation of sample.

Procedure

The well-mixed waste water sample was filtered under a vacuum through a membrane filter. 100mL of the waste water sample was transferred in a weighed evaporating dish. The sample was then evaporated to dryness on a steam bath. The evaporated waste water sample was dried for one hour in an oven at 180±2°C and cooled in a desiccator and then weighed. The drying was repeated until a constant weight was obtained or the weight loss was less than 0.5mg.

Calculation

Mg/L total filterable residue at 180°C = (A –B) x 1000 / C

Where:

A = weight of dried residue + dish

B = weight of dish

C = mL of filtrate used

3.6.0 Assement of Phycoremediation efficacies of *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa* on the nitrates and phosphates in tea, coffee and sugar effluents aganist WHO permissible stds.

The different waste waters were analyzed for Nitrates (micro kjeldahl apparatus) and phosphates (spectrophotometer at 420 nm) before and after algal inoculations (Sharma and Shakeel, 2013)

3.6.1 Nitrates (NO_3^-) according to cadmium reduction method

Before the nitrates were estimated especially from the coloured waste water Maghanga *et al.*,(2009) showed that it was important for the colour to be removed first through electro-coagulation method as shown in plate 3.7 and in the flow chart plate 3.8. Whereby the effluents (350 mL) drawn from each sampled factory was placed in a 500 mL beaker. Two steel plate electrodes, 137.5 mm height by 3.13 mm thickness by 50 mm width were used as both anode and cathode and suspended above the beaker containing the waste water sample. The electrodes were separated using a thick piece of carton so as to maintain the desired electrode spacing. Power was supplied by an AC to DC variable power supply unit and a variable a rheostat and ammeter were connected in series. An electrical shaker was used to shake the waste water sample at 100 rpm to maintain uniform shaking. The power and timer were switched on simultaneously and the current passing through the waste water sample was noted. Once the flocs started to form, the power was then switched off, the timer stopped and the time taken to form flocs was recorded and then the sampled was filtered to get the clear waste water sample for the estimation of nitrates.After each run, the electrodes were rinsed in 8 % sulfuric acid to avoid electrode fouling and passivation. Once the colour was removed the clear effluent was now subjected to nitrate and phosphate analysis using spectrophotometer as indicated in plate 3.9

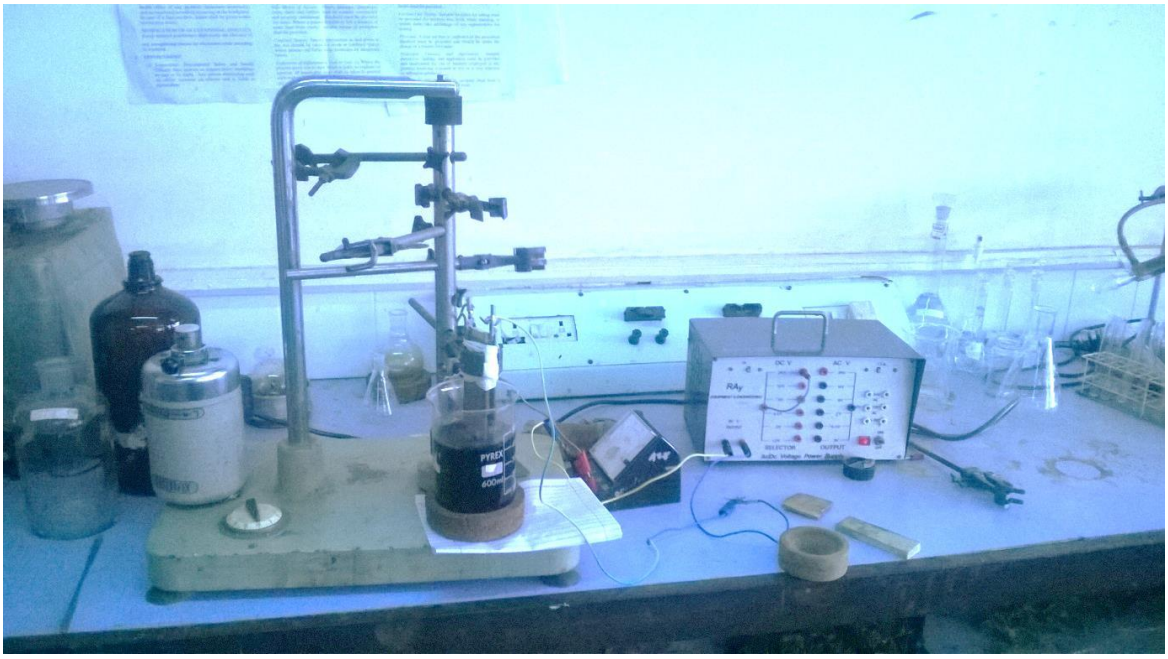


Plate 3.7: Electrocoagulation experimental method in progress. (photograph taken on 6.5.15 by A. Mbeke

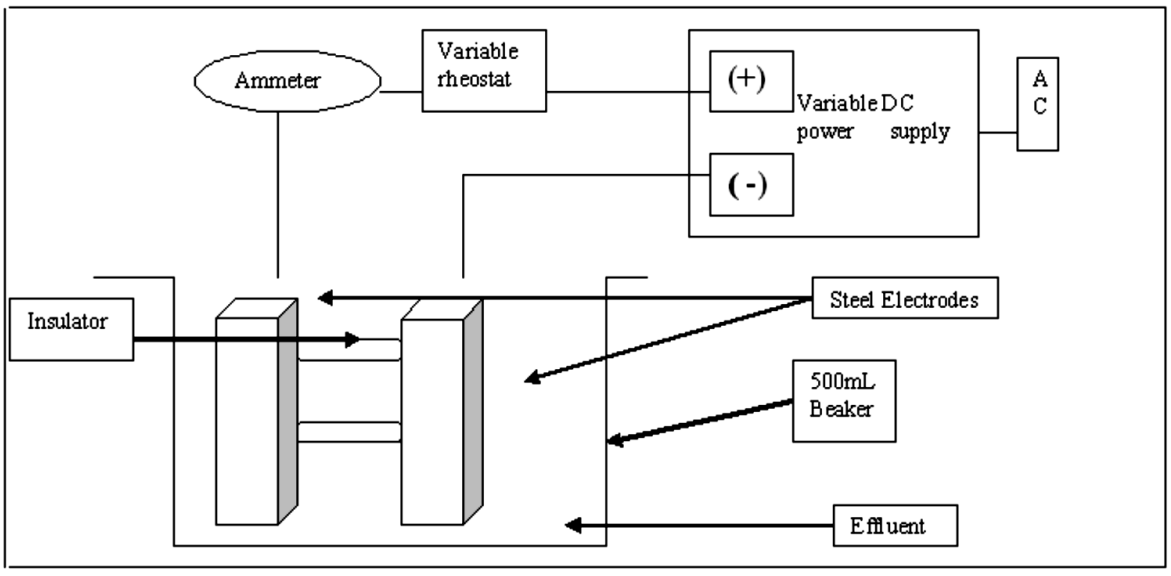


Plate 3.8: A sketch diagram of the Electrocoagulation method.

Nitrate (cadmium Reduction method) HR (0 to 30.0mg/L $N0_3^-$)analysis according to APHA 2005.

Principle

Nitrate was determined by measuring the absorbance at 220nm in sample containing 1ml of Hydrochloric acid (1N) in 100ml waste water sample. The concentration was calculated from graph of standard nitrate solution in range 1-11mg/L as N.The apparatus and equipment used in the analysis of nitrate as been indicated in appendix V.

Procedure;

The Hach program number 2530 N nitrate already stored for high range (HR) nitrate was selected from the spectrophotometer, the nitrate HR was displayed and the wave length 500nm automatically selected. The sample cell was then filled with 10ml of the waste water sample and the contents of Nitra Ver 5 Reagent powder pillow (the prepared sample) were added into the 10ml waste water sample and then the start time was pressed on and then the cell containing the mixture was shaken vigorously until the timer beeped after one minute. A deposit of unoxidized metal remained after the Nitra Ver 5 dissolved.

The10mg/l Nitrate Nitrogen standard solution was then used for standardization of the subsequent results and when the timer beeped the start key under start timer was pressed and a 5minute reaction period started. Amber color then develops if nitrate nitrogen was present. A second sample cell was again filled with 10ml of the waste water sample (the blank) and when the timer beeped from the machine the sample cell was then placed into the cell holder.

The spectrophotometer was then ‘zero’ in order to display 0.0mg/l NO_3^-then the prepared waste water samples were then placed into the cell holder and the light shield closed. The sample was then measured within one minute after timer beeped as shown in plate 3.9.The results in mg/l nitrate- nitrogen (NO_3^- - N) were then displayed (APHA 2005).

3.6.2 Phosphates (PO_4^-) analysis according to Andrew *et al*., (1995).

Principle: In the presence of a dilute orthophosphate solution, ammonium molybdate reacts under acidic conditions to form a heteropoly acid, molybdophosphoric acid. And In the presence of vanadium, a yellow vanadomolybdophosphoric acid is formed. The intensity of the yellow color is proportional to phosphate concentration.

Procedure

The pH of the 50ml sample if greater than 10 was first adjusted by adding 0.05ml (1drop) of phenolphthalein indicator to discharge the red color with 1m HCL before diluting to100ml then the excess color in the sample was removed to about 50ml with 200mg activated carbon in an Erlenmeyer flask for 5mins and then filtered to remove carbon. 35ml of sample containing 0.05 to 1.0mg phosphate was then put in a 50ml volumetric flask then 10ml vanadate; molybdate reagent was added then diluted to the mark with distilled water. After ten minutes or more the absorbance of the sample was measured at a wavelength of between 400-490nm versus the absorbance of the 35ml blank solution (Andrew *et.al.*,1995).

Plate 3.9: Phosphate, nitrate and COD estimation using a spectrophotometer (photograph taken on 6.5.15 by A. Mbeke).

3.6.3 Neubauer chamber method of cell enumeration according to APHA 2005;

Algal cell were counted by the use of a counting chamber just like any other body fluids however waste water samples containing algal cells were not diluted.

Procedure

The counting chamber was cleaned and wiped of any dust particle since this could cause unevenness in the lie of the cover glass on the slide, which could have altered the volume contained in the counting chamber the cover slip was then pressed on to the counting chamber until rainbow colors (Newton's rings) appeared. The ordinary cover glass are usually thin and therefore may alter the waste water volume of the counting chamber.

The chamber was then filled with the waste water sample containing the algal cells to be counted using a clean bore pipette with care being taken not to introduce air bubbles or to overfill the chamber then the chamber was placed in a petri dish containing a wet tissue for two minutes in order to prevent drying of the fluid when the cells were settling.The electric microscope x10 was swung into position and the iris diaphragm reduced, then the counting chamber was placed on the microscope stage and the cells and rulings of the chamber were brought into focus then,the cells overlapping the margins of the upper and right hand sides of the squares were counted, while those cells overlapping the margins of the lower and left hand sides of the squares were not considered.

The number of cells counted in $1mm^3$ of the fluid was calculated according to the area of the squares counted and the dilution factor of the fluid, the algal cell chamber and the cover glass were then cleaned and dried with a gauze and then stored in a safe box.Separate standard loop full inoculums of each algal species under investigation were subjected into the above cell counting procedure and on average all the algal species after calculation were found to be approximately 360,300 and 320 algal cells of *G.gleocapsa, C.vulgaris* and *S.salina* respectively. Then as shown in plate 3.10 a volume of 100mls of the sugar, tea and coffee waste waters from the respective sample sites were each separately inoculated with 10mls (dilution 1.1) of the respective algal species for the four replicate samples from each study site as follows.TT_C: Control for the waste water from all study site; TT_1: Waste water having *Chlorella vulgaris*; TT_2: Waste water having *Synechocystis salina;*TT_3: Waste water having *Gloeocapsa gelatinosa.*

Samples were collected after every 14 days for two occasions from the three counties and transported in a cool box to the laboratory for analysis of phosphate, nitrates, chemical oxygen demand, biological oxygen demand, TDS, pH and conductivity reductions.

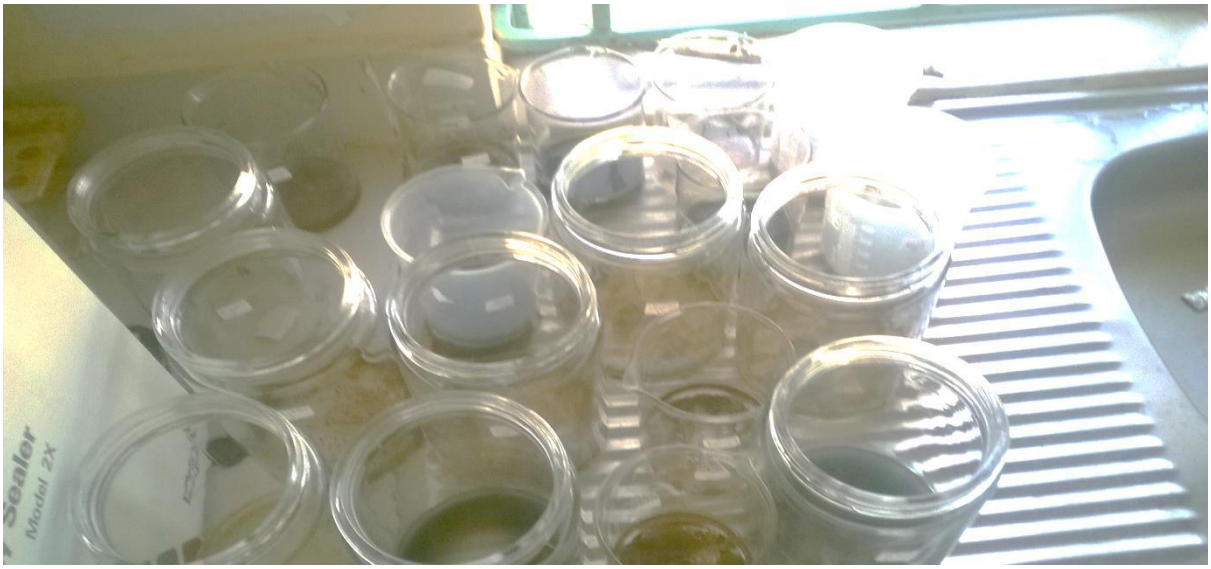


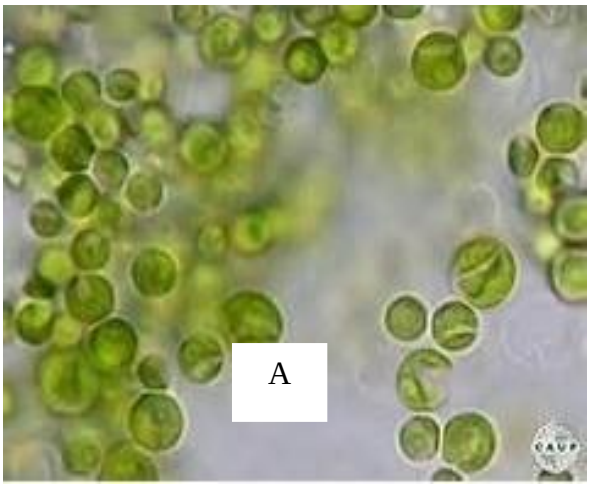
Plate 3.10: Waste water from different sampled locations inoculated with the specific algal species before incubation at the laboratory conditions (photograph taken on 6.5.15 by A. Mbeke).

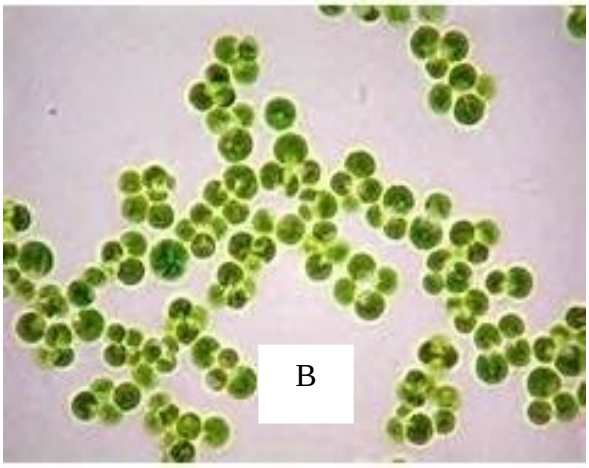
3.7.0 Comparison of the phycoremediation efficacies of the three algal species in the tea, sugar and coffee effluents.

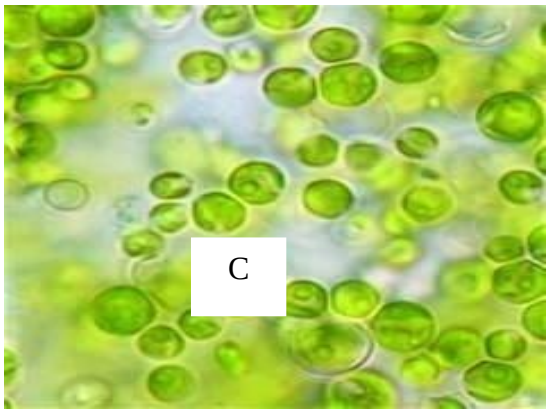
Response of microalgae on the waste water were measured by analyzing for the pH value using the pH meter while the nitrate and phosphate content were measured by taking optical density of waste water at different times using the Beer Lamberts law. The accumulation of free nutrients, ions and other chemical elements by microalgae were used to determine the phycoremediation efficacies of the three algal species used in the treatment of the different waste water. The difference between the initial and the second depiction reading gave an estimate of phycoremediation efficiency by micro-macroalgae.The different waste waters mixed with algae and the ones without the algae were analyzed in order to collect data on the pH, conductivity, TDS and also on the amounts of nitrates, phosphates and COD using spectrophotometer at 420 nm.

The amount of BOD was also measured using the BOD track machine.(Sharma and Shakeel,2013).

The algal species cells for use in the study were microscopically identified after isolation as indicated in plates 3.11, 3.12 and 3.13.







Legend

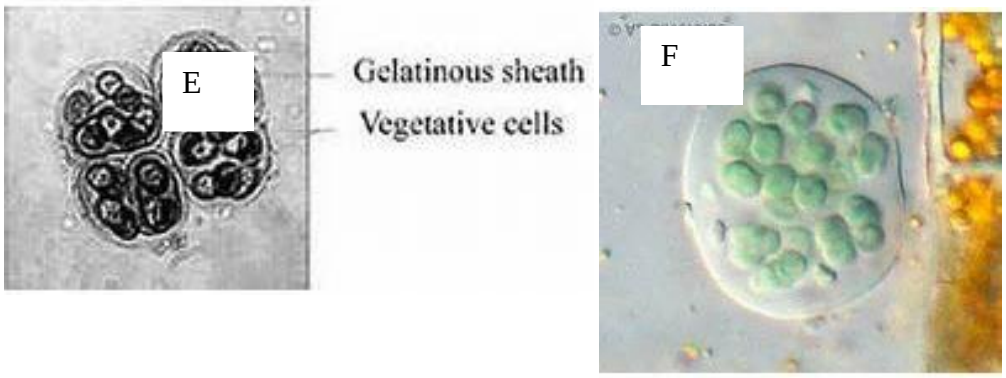
A –*C.vulgaris* x40

B – *C.vulgaris* x45

C – *C.vulgaris* x10

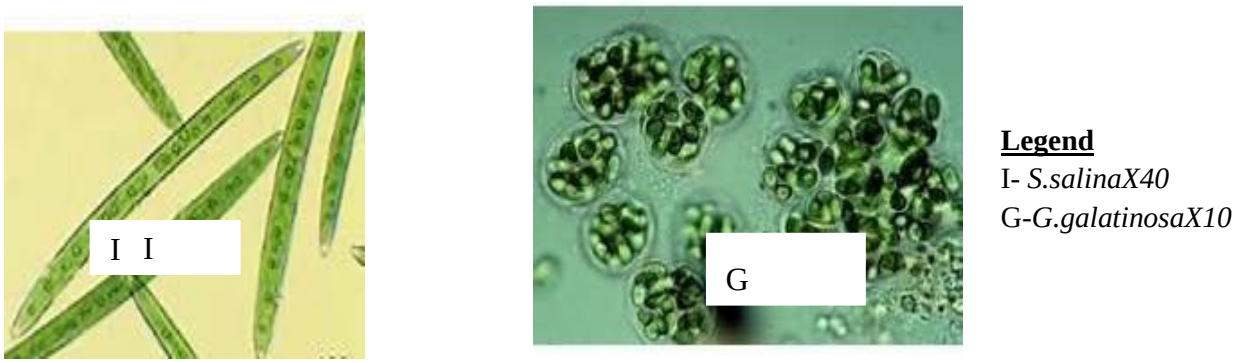
D – *C.vulgaris* x5

Plate 3.11: *Chlorella vulgaris* microscope photographs (21.6.15 by A.Mbeke)



Legend
E- *G.galatinosaX45*
F-*G.galatinosaX10*

Plate 3.12: *Gleocapsa gelatinosa* microscope photographs (21.6.15 by A.Mbeke)



Legend
I- *S.salinaX40*
G-*G.galatinosaX10*

Plate 3.13: *Synechocystis salina* microscope photograph x45 (21.6.15 by A.Mbeke)

Legend
I- S.salina X40
G- G.gelatinosaX10

3.8 Data validility and reliability.

The results from the study were valid and reliable because four replicate waste water samples were picked during each sampling period from each factory which had consented to the study, while all the samples were transported to the research laboratory in a cool box where they were analyzed immediately upon arrival at the laboratory depending with the specific requirements of the parameter to be analysed like the right incubation conditions. The research laboratory had a stand by generator incase of power interruptions. All the equipments used in the analysis of the waste water for the various physicochemical parameters under consideration were calibrated with standard controls of each parameter with the wavelengths set accordingly before each sample was analysed. Before and after each parameter was measured all the cuvettes used in the analysis were rinsed with de ionized water and wiped dry before being used again.

3.9 Data management and statistical analysis

After data collection, entry was done using Microsoft Excel, 2000 for Windows and later exported to STATISTICA V.8.0 and restructured for re-entry and analysis. Descriptive statistics; means, percentages, standard deviations and standard errors were used to summarize the physio-chemical parameters of coffee, tea and sugar waste waters from the study sites (factories).

Independent t-test was used to assess and compare the levels of pH,COD,BOD, TDS, and conductivity parameters including nitrates and phosphates in all the periods, that is day 0, day 5, day 10 and day 15 in the algae treated effluents of tea, coffee and sugar factories with WHO permissible standards. T-test was used since the replicates were 4 ($n<30$) and independent. One way Analysis of variance (ANOVA) test were used to compare pollutants removal efficacy of the three algae species (*S.salina, C.vulgaris and G.gelatinosa*) in coffee tea and sugar affluent respectively. In all the analysis, $p<0.05$ was considered significant and interpretations done appropriately. Results were presented in form of tables and figures.

CHAPTER FOUR: RESULTS

Introduction

The influent and effluent values of all parameters from the study sites were depicted in appendix XI. There was no significant difference ($p>0.05$) between influent and effluent for all the parameters in the three types of waste water indicating a likelihood association of waterborne diseases as shown in Table 4.1.

Table 4.1 Deviations of Effluent and Influent Physicochemical Parameters in coffee, sugar and tea

EFFLUENT TYPE	PARAMETER	WHO STDS	OBSERVED		DEVIATION	P-VALUE
			INFLUENT	EFFLUENT		
TEA	TDS	2000	559.88	500.13	59.8	0.5044
	PH	6.5-8.5	7.21	7.8	-0.59	0.0969
	Nitrate	10	4.93	3.97	0.96	0.2718
	phosphate	5	74.52	68.16	6.36	0.4539
	BOD	40	589.75	470.44	119.31	0.3202
	COD	250	1984.81	1747.25	237.56	0.54041
	Conductivity	500	902.94	806.44	96.50	0.5038
COFFEE	TDS	2000	734.88	734.88	0	1.0000
	PH	6.5-8.5	6.50	6.61	-0.11	0.8313
	Nitrate	10	12.25	12.0	0.25	0.9523
	phosphate	5	4.42	4.06	0.36	0.8778
	BOD	30	213.0	211.0	2.00	0.9902
	COD	250	3459.0	3454.5	4.5	0.9987
	Conductivity	500	1185.25	1184.38	0.88	0.9987
SUGAR	TDS	2000	308.45	234.05	74.04	0.8624
	PH	6.5-8.5	5.35	5.0	0.35	0.8980
	Nitrate	10	18.6	15.95	2.65	0.8776
	Phosphate	5	20.24	17.25	2.99	0.9175
	BOD	30	2496.5	2142.0	354.5	0.8992
	COD	250	3731.0	3372.5	358.5	0.9339
	Conductivity	500	493.0	372.5	120.5	0.8634

The three effluents from coffee, tea and sugar were each inoculated with a set of *C.vulgaris, S.salina and G.gelatinosa* species of algae and phycoremediation was observed in all the sets. Before inoculation each effluent was determined for TDS, pH, nitrate, phosphate, COD and conductivity and the values indicated as the initial concentrations shown in each day zero and used subsequently as the controls. However BOD was determined after 5 days as BOD_5.

4.1 Phycoremediation efficacy of *C.vulgaris, S.salina and G.gelatinosa* on the physico-chemical parameters of coffee, tea and sugar effluents

In the phycoremediation of TDS of coffee, tea and sugar effluents the concentration values were shown in table 4.2. The TDS concentration values were noticed to reduce from day 0 to day 15 upon phycoremediation with the highest phycoremediation effect noticed between day 1 and day 5 and stabilizing at day 10 as shown in Figures 4.1, 4.2 and Figure 4.3.

No significance difference (p=0.1137; p=0.1830) in TDS phycoremediation efficacy was noted for *S.salina* and *G.gelatinosa* respectively between day 1 and day 5 in coffee effluent but it was significantly different in *C.vulgaris* with p=0.0388 (Appendix X). In tea effluent there was a significant difference in TDS phycoremediation efficacy between day 1 and day 5 for the *C.vulgaris, S.salina* and *G.gelatinosa* algal species (p = 0.0001) while in sugar effluent the significant differences noted were p = 0.0086, p = 0.0026 and p=0.0066 for the *C.vulgaris, S.salina* and *G.gelatinosa* respectively (Appendix X). Between day 5 and day 10 in the coffee effluent there was no significant difference in TDS phycoremediation efficacy in *S.salina, C.vulgaris* and *G.gelatinosa* (p=0.6330, p= 0.7522 and p=0.71258) while between day 10 and day 15 there was a non significant difference with p=1.0000 in all the three algal species used.

In tea effluent there was a significant difference between day 1 and day 5 of p=0.0000l, p=0.0000 and p=0.00006 for *S.salina, C.vulgaris* and *G.gelatinosa* respectively.However non significant difference in phycoremediation was observed between day 5 and day 10, p=0.27743, p=0.12770 and 0.67433 for the *S.salina, C.vulgaris* and *G.gelatinosa.* However between day 10 and day 15 the phycoremediation efficacy of TDS by the three algal species was noted to have a uniform non significant difference of p =1.0000. In sugar effluent the TDS phycoremediation efficacy between 1 day and day 5 was significantly different with p=0.0086, p =0.00260 and p=0.00662 for the *S.salina, C.vulgaris* and *G.gelatinosa* respectively. Between day 5 and day 10 the phycoremediation of TDS was also significantly different with *S.salina* p = 0.01820, *C.vulgaris* p=0.00907 and *G.gelatinosa* having a p=0.00021.However the phycoremediation efficacy was not significantly different between day 10 and 15 with p =1.0000 for *S.salina, C.vulgaris* and *G.gelatinosa* as shown in Figure 4.3 and in Appendix X.

Table 4.2 Phycoremediation efficacies of *C.vulgaris, S.salina* and *G.gelatinosa* on the TDS of coffee, tea and sugar effluent

EFFLUENT TYPE	PAR	DAY	*S.salina*	% removal	*C. vulgaris*	% removal	*G. gelatinosa*	% removal
COFFEE	Control	Day0	734.875±234.52	0%	734.875±234.52	0%	734.875±234.52	0%
	TDS Mg/L	Day1	734.875±234.52	0%	734.875±234.52	0%	734.875±234.52	0%
		Day5	306.25±97.795	58%	183.875±58.655	75%	367.625±117.21	50%
		Day10	243.125±84.607	67%	158.875±50.849	79%	306.875±111.228	58%
		Day15	243.125±84.607	67%	158.875±50.849	79%	308.875±111.228	58%
TEA	Control	Day0	505.875±58.987	0%	505.875±58.987	0%	505.875±58.987	0%
	TDS Mg/L	Day1	505.875±58.987	0%	505.875±58.987	0%	505.875±58.987	0%
		Day5	169.1937±25.056	67%	105.0187±12.475	79%	192.5188±32.818	62%
		Day10	131.9375±22.506	74%	81.8125±7.988	84%	174±28.764	66%
		Day15	131.9375±22.506	74%	81.8125±7.988	84%	174±28.764	66%
SUGAR	Control	Day0	474.5±12.5	0%	474.5±12.5	0%	474.5±12.5	0%
	TDS Mg/L	Day1	474.5±12.5	0%	474.5±12.5	0%	474.5±12.5	0%
		Day5	285.5±12.5	40%	198.5±6.5	58%	320.5±1.5	32%
		Day10	177±8	63%	121.5±3.5	74%	196±1	59%
		Day15	177±8	63%	121.5±3.5	74%	198±1	59%

Note: Day 0 represented the control values.

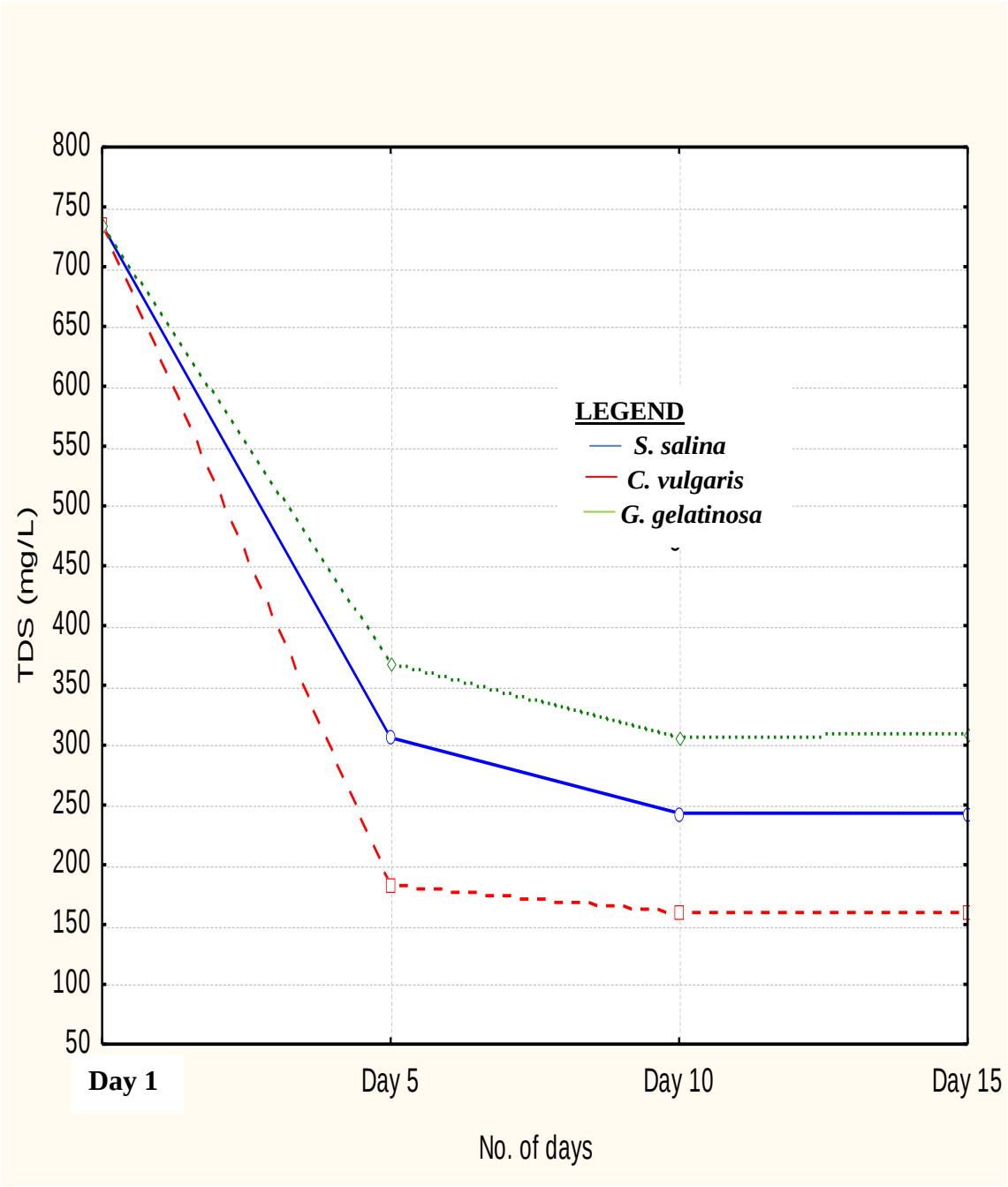


Figure 4.1 Phycoremediation efficacies of *C .vulgaris, S.salina* and *G.gelatinosa* on TDS of Tea effluent

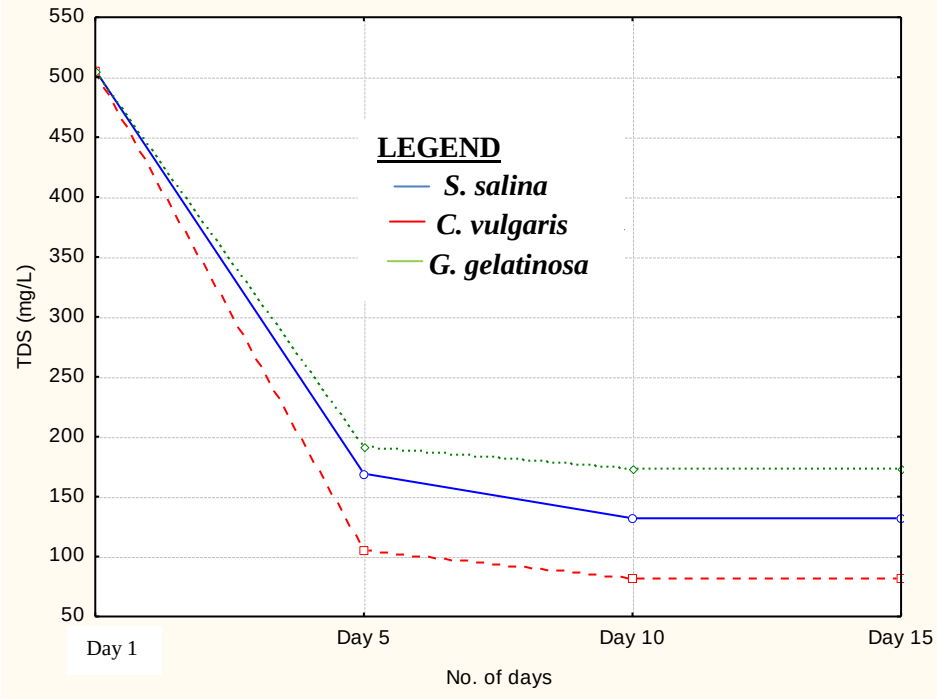


Figure 4.2 Phycoremediation efficacies of *C .vulgaris, S.salina* and *G.gelatinosa* on TDS of sugar effluent.

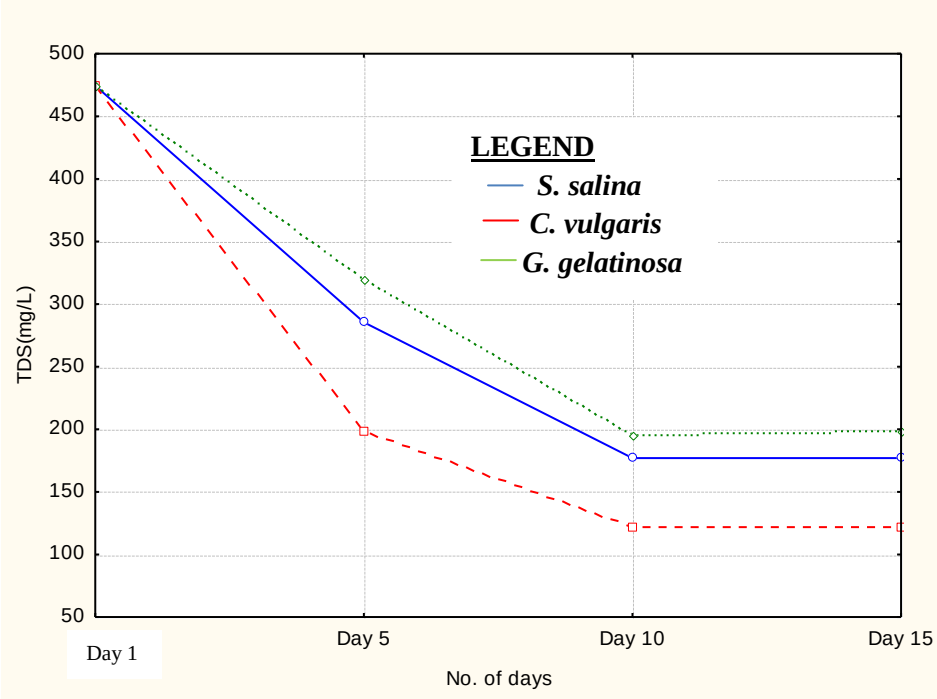


Figure 4.3 Phycoremediation efficacies of *C .vulgaris, S.salina* and *G.gelatinosa* on TDS of Coffee effluent.

The phycoremediation effect of *S.salina, C.vulgaris* and *G.gelatinosa* on the pH of coffee, tea and sugar effluents was shown in table 4.3. The pH of all the three effluents upon phycoremediation was reported to increase between day 1 and day 5 and then decreased in day 10 in all the effluents before stabilizing between day 10 and day 15 where no further increase or decrease was recorded as shown in Figures 4.4, 4.5 and Figure 4.6. Between day 1 and day 5 there was no significant pH phycoremediation difference noted in the coffee effluent treated with *S.salina* and *C.vulgari* (p-value=0.10761 and p-value=0.17093) while *G.gelatinosa* had a significant phycoremediation difference of p-value= 0.03144.

Between day 5 and day 10 there was no significant difference in phycoremediation of coffee effluent using the *S.salina, C.vulgaris* and *G.gelatinosa* (p-value=0.97539, p-value= 0.97374 and p-value=1.0000).While between day 10 and day 15 no significant difference of p-value=1.0000 was noted in both *C.vulgaris* and *G.gelatinosa* and another non significant difference of p-value=0.97490 in the *S.salina* (see Appendix X and Figure 4.4).

Considering the pH of the tea effluent phycoremediation shown in table 4.3 and summarized in Figure 4.5and in Appendix X, it was noted that between day 1 and day 5 there was significant pH phycoremediation difference noted in the tea effluent treated with *S.salina, C.vulgaris and G.gelatinosa* (p-value=0.000210, p-value= 0.000192 and p-value=0.000191) respectively. While between day 5 and day 10 there was significant differences in pH phycoremediation of tea effluent using the *S.salina and C.vulgaris* (p-value=0.02763, p-value= 0.00656) and no significant difference using *G.gelatinosa* p-value=0.05041. Between day 10 and day 15 no significant differences in phycoremediation (p-value=0.96099, p-value= 0.98057 and p-value=0.9181) was noted in *S.salina, C.vulgaris* and *G.gelatinosa* respectively.

In the sugar effluent there was no significant phycoremediation efficacy difference recorded between day 1 and day 5 with p-value=0.08074,p-value=0.05160 and p-value=0.05705 for the *S.salina, C.vulgaris* and *G.gelatinosa* respectively.Between day 5 and day 10 still there was no phycoremediation difference noted with *S.salina* p-value=0.764298,*C.vulgaris* p-value=0.31175 and *G.gelatinosa* p-value=0.89517. While between day 10 and day 15 both *S.salina* and *C.vulgaris* had no significant phycoremediation difference (p-value=1.0000) with *G.gelatinosa* having no significant difference (p-value= 0.9098) as shown in Figure 4.6 (Appendix X).

Table 4.3 Phycoremediation efficacies of *S.salina, C.vulgaris* and *G.gelatinosa* on the pH of coffee, tea and sugar effluents.

EFFLUENT TYPE	PARAMETER	DAY	*S. salina*	*C. vulgaris*	*G. gelatinosa*
COFFEE	control	Day0	6.5±0.378	6.5±0.378	6.5±0.378
	pH	Day1	6.5±0.378	6.5±0.378	6.5±0.378
		Day5	7.313±0.284	7.162±0.26	7.65±0.298
		Day10	7.3±0.279	7.175±0.267	7.65±0.298
		Day15	7.288±0.273	7.175±0.267	7.65±0.298
TEA	control	Day0	7.711±0.2941	7.711±0.2941	7.711±0.2941
	pH	Day1	7.711±0.2941	7.711±0.2941	7.711±0.2941
		Day5	9.017±0.0967	9.024±0.0944	9.04±0.106
		Day10	8.698±0.098	8.643±0.0901	8.731±0.1082
		Day15	8.691±0.0991	8.6394±0.0899	8.716±0.1049
SUGAR	control	Day0	6.55±0.45	6.55±0.45	6.55±0.45
	pH	Day1	6.55±0.45	6.55±0.45	6.55±0.45
		Day5	8.25±0.25	8.5±0.1	8.45±0.15
		Day10	8.15±0.15	8.35±0.05	8.4±0.3
		Day15	8.15±0.15	8.35±0.05	8.35±0.25

Note: Day 0 represented the control values.

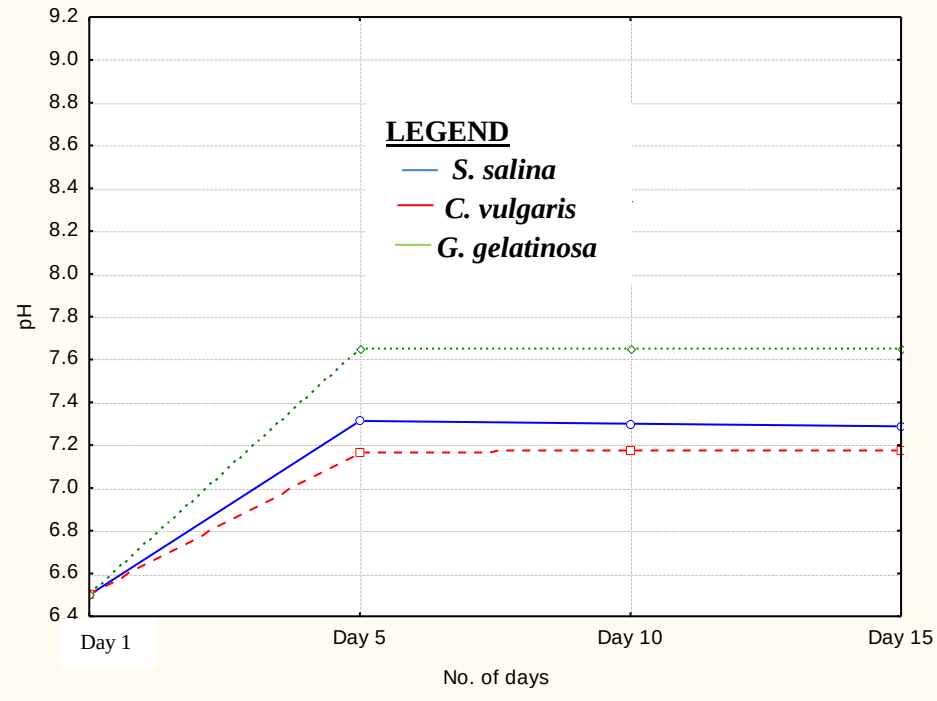


Figure 4.4 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on pH of coffee effluent

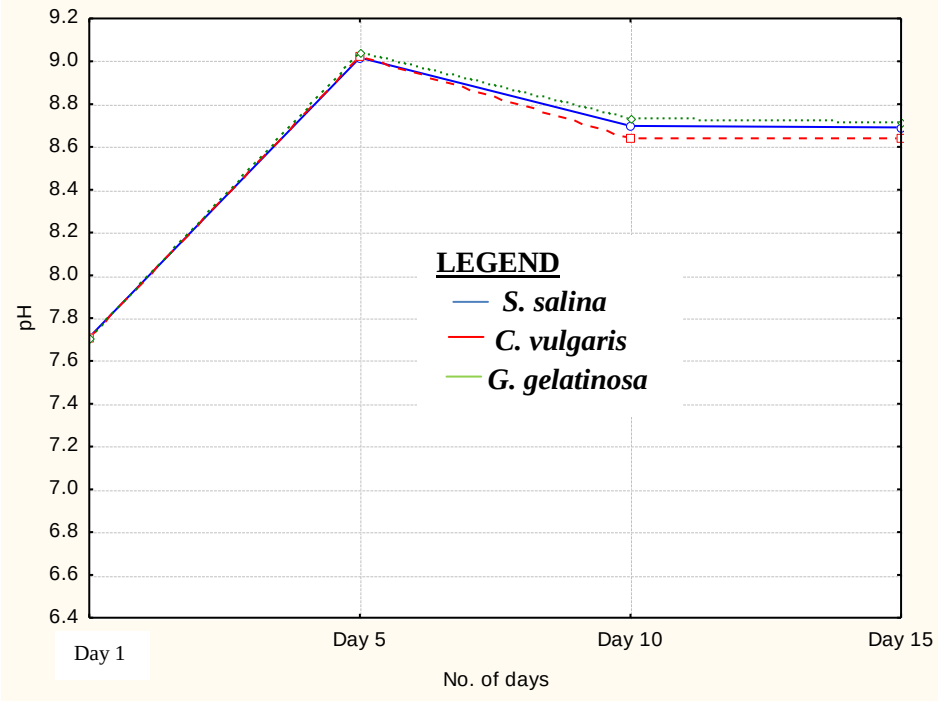


Figure 4.5 Phycoremediation efficacy of *C .vulgaris, S.salina,* and *G.gelatinosa* on pH of tea effluent

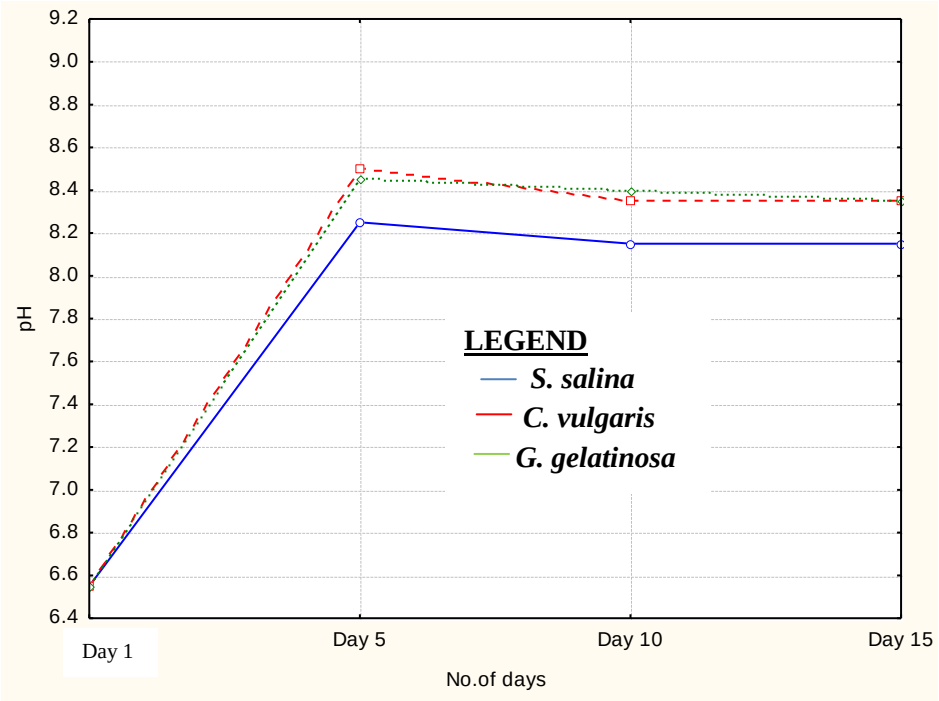


Figure 4.6 Phycoremediation efficacy of *C .vulgaris, S.salina and G.gelatinosa* on pH of sugar effluent

Table 4.4 shows the effect of *S.salina, C .vulgaris and G.gelatinosa* on the nitrate content present in coffee, tea and sugar effluents. In coffee effluent the highest phycoremediation and reduction effect on the mean nitrate was noticed between day 1 and day 5 whereby *S.salina, C .vulgaris and G.gelatinosa* had significant difference in phycoremediation efficacy of p-value=0.000133, p-value=0.000063 and p-value=0.001101 respectively. Between day 5 and day 10 the phycoremediation efficacy of nitrate were not significantly different with *S.salina* (p-value=0.06754), *C.vulgaris* (p-value=0.18698) and *G.gelatinosa* (p-value=0.102581). While between day 10 and day 15 the phycoremediation efficacy were also not significantly different with p-value=0.55886,p-value=0.864810 and p-value=0.72535 for *S.salina, C .vulgaris* and *G.gelatinosa* respectively (Appendix X). The phycoremediation success was 75%,76% and 65% for the *S.salina, C .vulgaris* and *G.gelatinosa* respectively as shown in Table 4.4 and in Figure 4.7.

However in the phycoremediation of nitrates in tea effluent using *S.salina* its effect had a statistical significant difference between day 1 and day 5 (p= 0.005140) and no significant difference between day 5 and day 10 and between day 10 and day 15 (p-value=0.195718 and p-value=1.00000) respectively. The *C.vulgaris* had a significant difference in phycoremediation efficacy between day 1 and day 5 and no significant difference between day 5 and day 10 and between day 10 and day 15 (p-value=0.000616, p-value=0.366687 and p-value=0.312991) respectively. While the *G.gelatinosa* had no significant difference in phycoremediation efficacies (p-vaue=0.055811) between day 1 and day 5 and p-value=0.45822 between day 5 and day 10 while between day 10 and day 15 no significant difference in phycoremediation was reported (p-value=0.88480) see Appendix X and Figure 4.8.

In the phycoremediation efficacy of nitrates in sugar effluent using *S.salina* there was a significant difference (p-value=0.037860) and a non significant difference (p-value=0.224120 and p-value=0.859972) between day 1 and day 5; between day 5 and day 10 and between day 10 and day 15 respectively. The *C.vulgaris* had a significant difference in phycoremediation efficacy (p-value=0.039014) between day 1 and day 5, while between day 5 and day 10 and between day 10 and day 15 there were no significant differences in phycoremediation (p-value=0.285431 and p-value=1.00000). The *G.gelatinosa* had a significant difference in phycoremediation efficacy (p-value=0.035648) between day 1 and day 5. While between day 5 and day 10 and between day 10 and day 15 there were no significant differences in nitrate phycoremediation efficacies (p-value=0.18889 and p-value=1.00000) (Appendix X). The

nitrate reduction trends have been shown in Table 4.4 and in Figures 4.7;4.8 and 4.9 respectively.

Table 4.4 Phycoremediation efficacies of *C.vulgaris, S.salina* and *G.gelatinosa* on the nitrates of coffee, tea and sugar effluent

EFFLUENTTYPE	PARAMETER	DAY	*S. salina*	*% Removal*	*C. vulgaris*	*% Removal*	*G. gelatinosa*	*% removal*
	Control	Day0	21.375±2.129	0%	21.375±2.129	0%	21.375±2.129	0%
		Day1	21.375±2.129	0%	21.375±2.129	0%	21.375±2.129	0%
		Day5	8.575±1.231	60%	7.675±1.187	64%	11.012±1.372	49%
	NITRATES	Day10	5.838±0.628	73%	5.688±0.801	76%	8.088±0.96	62%
COFFEE	Mg/L	Day15	5.275±0.699	75%	5.5±0.726	76%	7.588±1.012	65%
	control	Day0	4.119±0.5463	0%	4.106±0.5451	0%	4.106±0.54	0%
		Day1	4.119±0.5463	0%	4.106±0.5451	0%	4.106±0.54	0%
		Day5	2.274±0.2735	44%	1.798±0.2587	56%	2.794±37	32%
	NITRATES	Day10	1.809±0.2213	56%	1.819±3.2862	56%	2.426±0.3199	41%
TEA	Mg/L	Day15	1.809±0.2213	56%	1.4388±0.2307	66%	2.361±0.3152	42%
	control	Day0	28±2	0%	28±2	0%	28±2	0%
		Day1	28±2	0%	28±2	0%	28±2	0%
		Day5	10±3	64%	9.25±3.25	67%	11.5±2.5	59%
	NITRATES	Day10	4.5±1	84%	4.5±0.5	84%	6.5±0.5	77%
SUGAR	Mg/L	Day15	4.25±0.75	84%	4.5±0.5	85%	6.5±0.5	77%

Note: Day 0 represented the control values.

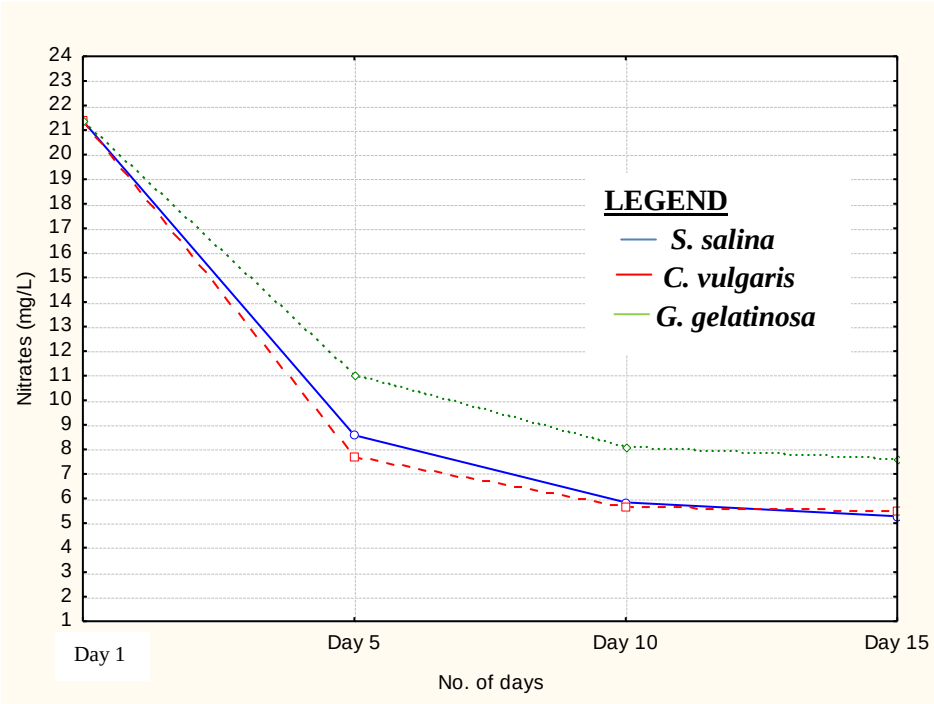


Figure 4.7 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on nitrates of coffee effluent

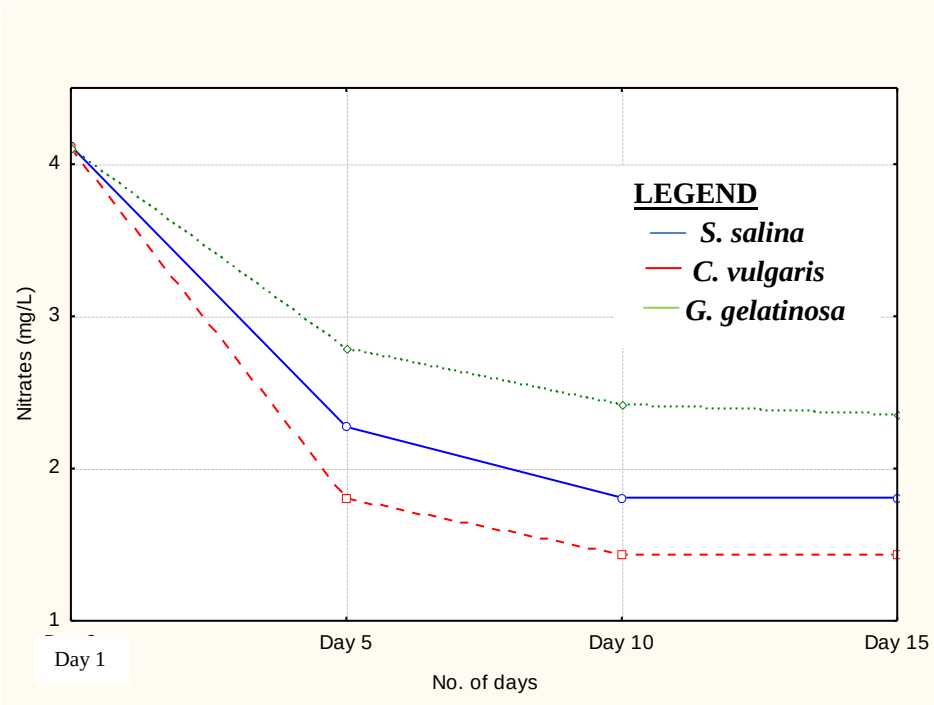


Figure 4.8 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on nitrates of tea effluent

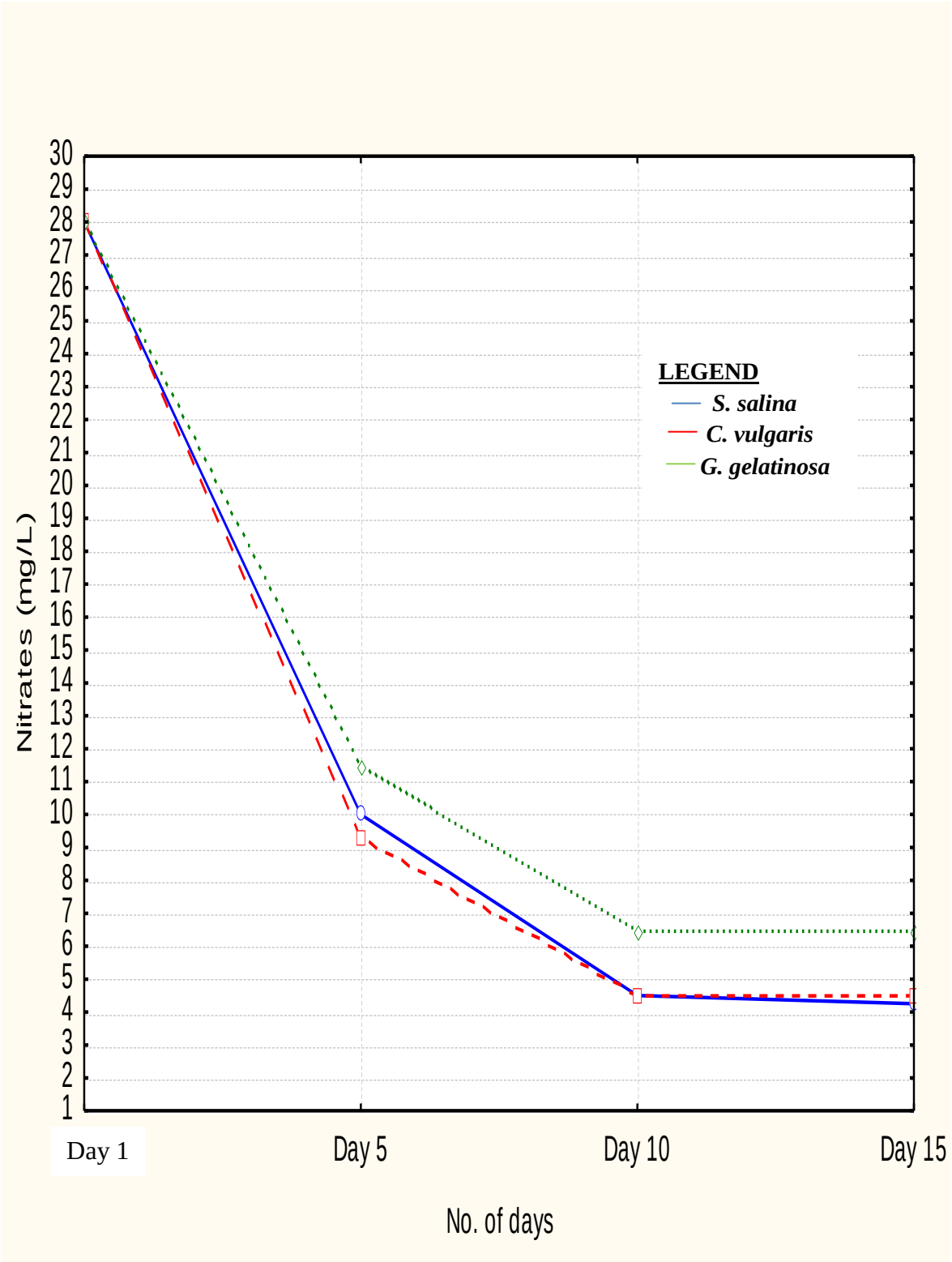


Figure 4.9 Phycoremediation efficacy of *C .vulgaris, S.salina and G.gelatinosa* on nitrates of sugar effluent.

In Table 4.5 the phycoremediation efficacy of *C.vulgaris, S.salina* and *G.gelatinosa* was noted on the phosphate content of coffee,tea and sugar effluents whereby between day 1 and day 5 a significant difference in phycoremediation of phosphate content of the tea effluent using *S.salina,C.vulgaris* and *G.gelatinosa* (p-value=0.000004,p-value=0.000002 and 0.00002) respectively.While between day 5 and day 10 the phycoremediation efficacies were not significantly different (p-value=0.76744,p-value=0.825817 and p-value=0.65846) for *S.salina,C.vulgaris* and *G.gelatinosa* respectively.Non significant differences were also noted in the phycoremediation of phosphates in tea effluent between day 10 and day 15 with *S.salina* (p-value=0.972828), *C.vulgaris* (p-value=0.953244) and *G.gelatinosa* (p-value=0.983621) as shown in Figure 4.11 and in Appendix X.

The phycoremediation of phosphates in sugar effluent showed no significant difference between day 1 and day 5 (p-value= 0.111094, p-value= 0.099315 and p-value=0.139732) for *S.salina, C.vulgaris* and *G.gelatinosa* respectively. While between day 5 and day 10 *S.salina, C.vulgaris* and *G.gelatinosa* had also no significant differences (p-value=0.295639, p-value= 0.428452 and p-value= 0.380414) respectively. Between day 10 and day 15 non significant difference (p-value= 0.947514) using *S.salina* and p-value= 1.00000 for both *C.vulgaris* and *G.gelatinosa* were recorded as shown in Figure 4.12, and in Appendix X.

However phosphate phycoremediation efficacy in coffee effluent was not significantly different between day 1 and day 5, day 5 and day 10 and also between day 10 and day 15 using *S.salina, C.vulgaris* and *G.gelatinosa* ($p>0.05$) as shown in Figure 4.10 and in Appendix X.

Table 4.5 Phycoremediation efficacy of *C.vulgaris, S.salina* and *G.gelatinosa* on the phosphates of coffee, tea and sugar effluent

EFFLUENTTYPE	PARAMETER	DAY	*S. Salina*	% removal	*C. vulgaris*	% removal	*G. gelatinosa*	% removal
	Control	Day0	4.419±1.784	0%	4.419±1.784	0%	4.419±1.784	0%
		Day1	4.419±1.784	0%	4.419±1.784	0%	4.419±1.784	0%
		Day5	2.511±1.345	43%	2.05±1.153	55%	2.932±1.463	34%
	PHOSPHATES	Day10	2.04±1.097	55%	1.644±0.899	64%	2.383±1.103	45%
COFFEE	Mg/L	Day15	2.039±1.097	55%	1.641±0.897	64%	2.382±1.103	45%
	Control	Day0	69.196±5.789	0%	69.196±5.789	0%	69.071±5.764	0%
		Day1	69.196±5.789	0%	69.196±5.789	0%	69.071±5.764	0%
		Day5	31.6±3.360	54%	29.853±3.176	57%	34.922±3.530	50%
	PHOSPHATES	Day10	30.208±3.238	57%	28.87±3.086	58%	32.754±3.335	53%
TEA	Mg/L	Day15	30.05±3.247	57%	28.6125±3.073	59%	32.656±3.369	53%
	Control	Day0	5±0.9	0%	5±0.9	0%	5±0.9	0%
		Day1	5±0.9	0%	5±0.9	0%	5±0.9	0%
		Day5	2.35±0.35	52%	2.05±0.45	58%	2.8±0.2	44%
	PHOSPHATES	Day10	1.55±0.45	68%	1.35±0.55	72%	2.35±0.35	52%
SUGAR	Mg/L	Day15	1.50±0.5	70%	1.35±0.55	72%	2.35±0.35	52%

Note: Day 0 represented the control values.

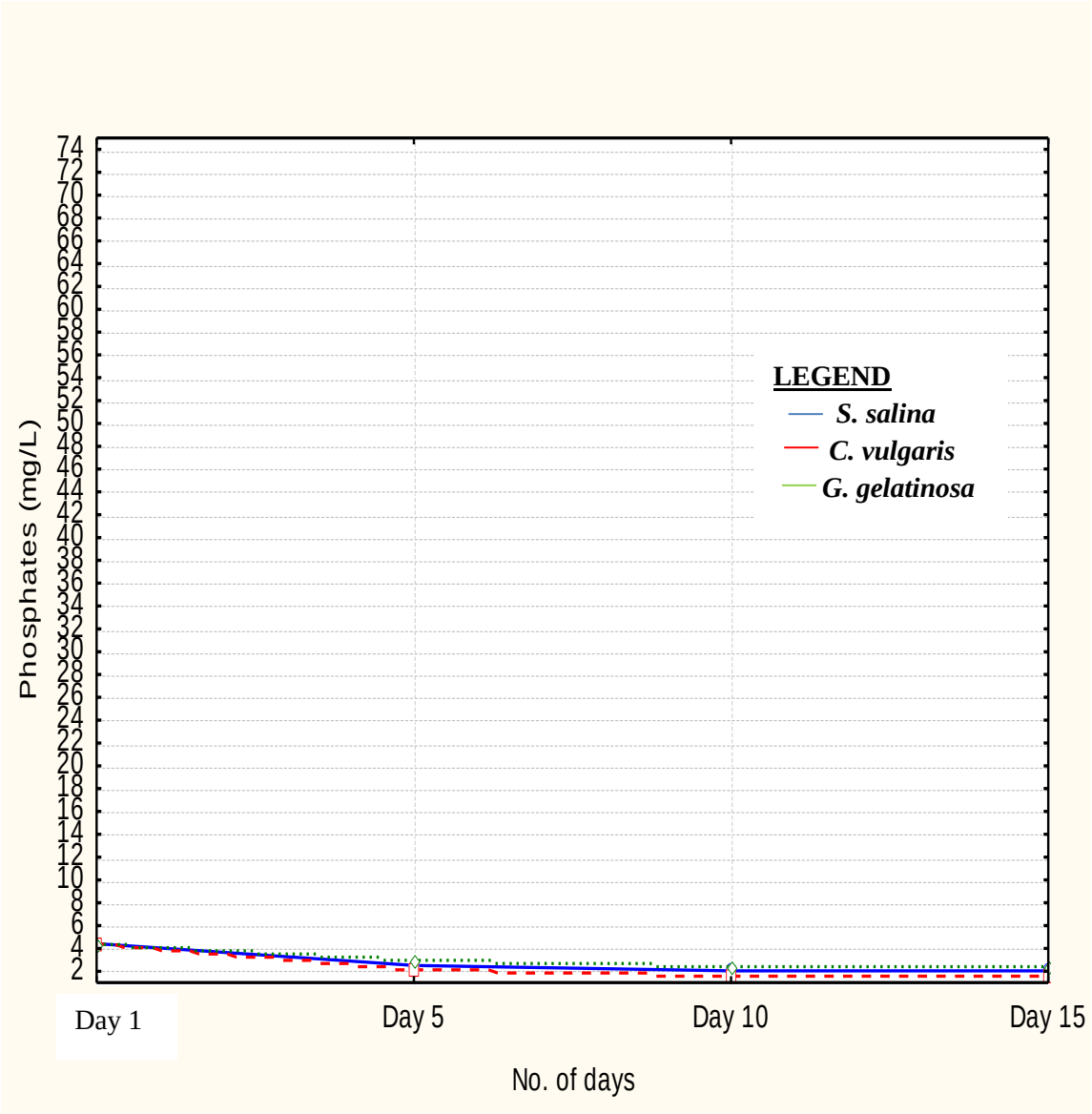


Figure 4. 10 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on phosphates of coffee effluent

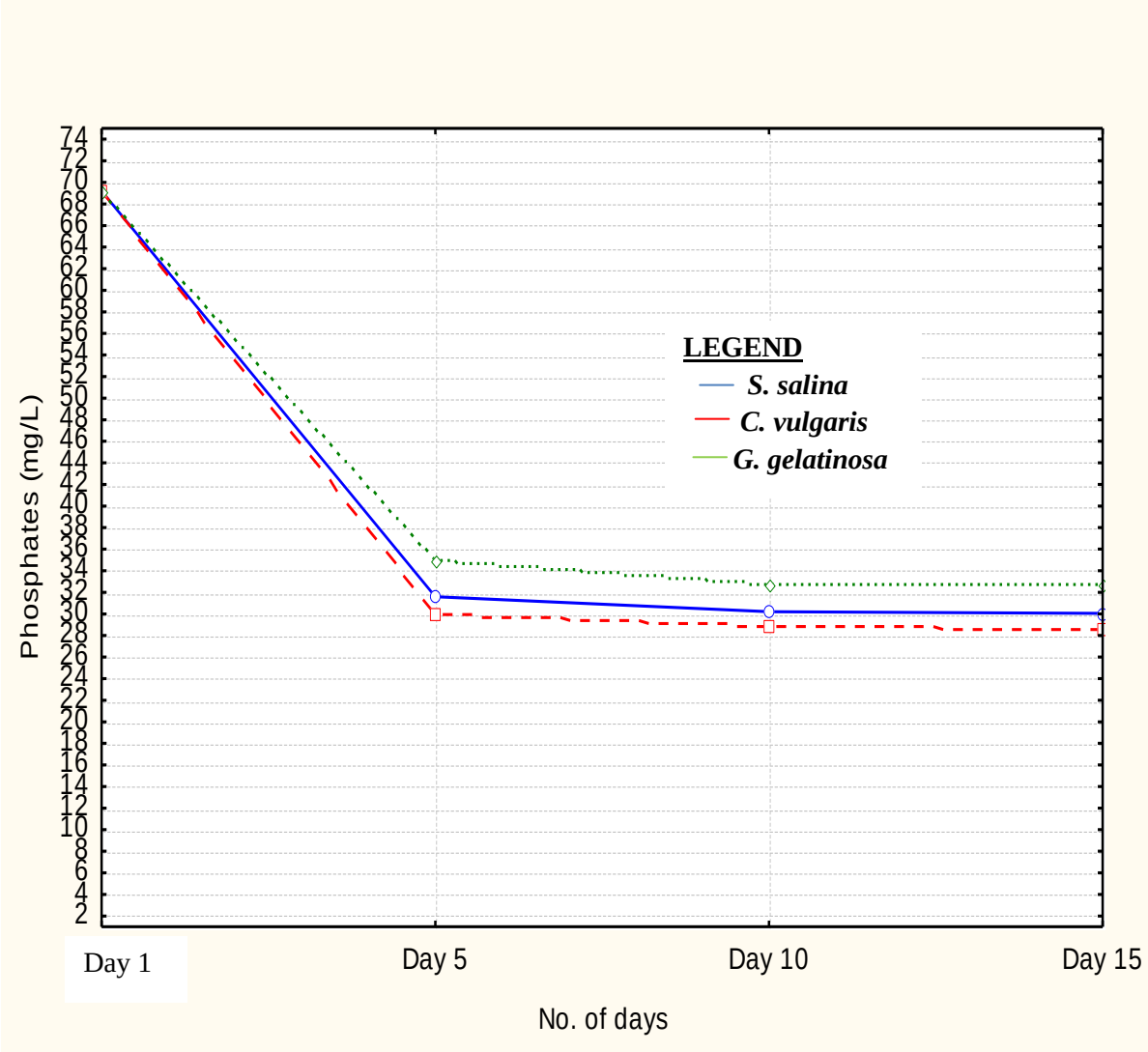


Figure 4. 11 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on phosphates of tea effluent

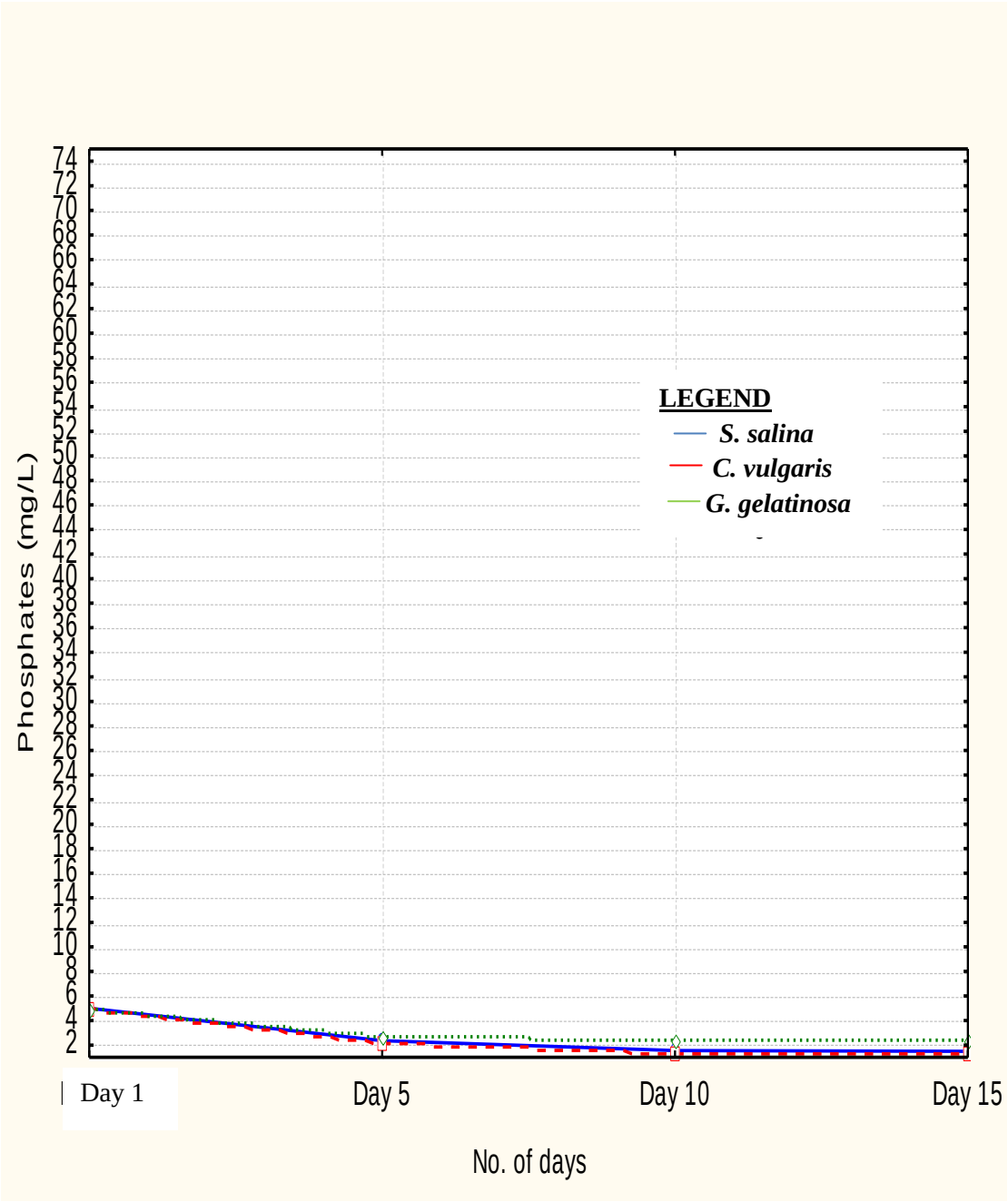


Figure 4.12 Phycoremediation efficacy of *C.vulgaris, S.salina* and *G.gelatinosa* on phosphates of sugar effluent

Phycoremediation effect on BOD in the coffee, tea sugar effluents were recorded and (Table 4.6). The BOD was measured after five days of incubation hence BOD_5. In figure 4.13, *C .vulgaris, S.salina,* and *G.gelatinosa* were shown to reduce the BOD levels in coffee effluent from around 900mg/l to about 200mg/l, 400mg/l and 520mg/l between days 5 and 10 for the three species respectively.There was no change noticed between day 10 and day 15 in all the species. There was no significant difference in phycoremediation efficacy of the BOD content between day 5 and day 10 (p-value= 0.789961, p-value= 0.288774 and p-value=

0.289247) for *S.salina, C.vulgaris* and *G.gelatinosa* respectively. While between day 10 and day 15 no significant differences in phycoremediation (p-value= 1.0000) was noted with *S.salina* and *G.gelatinosa* and with *C.vulgaris* (p-value= 0.998777) as shown in Figure 4.13 (see Appendix X) whereby the algal species had almost a similar phycoremediation reduction trend.

The tea effluent phycoremediation (Figure 4.14) indicated that there was a phycoremediation effect of BOD from about 1200mg/l to 200mg/l between day 5 and 10 for all the three species. However no change was noted between day 10 and 15 for *C .vulgaris, S.salina,* and *G.gelatinosa* respectively. Between day 5 and day 10 there was a BOD non significant differences in phycoremediation efficacy using *S.salina* (p-value= 0.07993), *C.vulgaris* (p-value= 0.066158) and *G.gelatinosa* (p-value= 0.174205). Whereas between day 10 and day 15 the phycoremediation efficacies for the three species were (p-value= 1.00000) as shown in Appendix X.

The BOD of the sugar effluent (figure 4.15) reduced from a high of about 650mg/l to 430mg/l, 650mg/l to 480mg/l and 650mg/l to 500mg/l between day 5 and 10 after treatment with *C .vulgaris, S.salina* and *G.gelatinosa* respectively. Between day 10 and 15 there was no phycoremediation change noticed. However there was a significant difference in BOD reduction in the sugar effluent between day 5 and day 10 with P-value=0.030666 and P=0.000905 for *S.salina* and *C.vulgaris* respectively with no phycoremediation significant difference noticed with *G.gelatinosa* (p=0.104897) while between day 10 and day 15 a non significant difference in BOD phycoremediation efficacy (p-value=1.00000) was noted in the three algal species as shown in Appendix X

Table 4.6 Phycoremediation efficacy of *C.vulgaris, S.salina* and *G.gelatinosa* on the BOD of coffee, tea and sugar effluent

EFFLUENT TYPE	PAR	DAY	*S. Salina*	% Removal	*C. vulgaris*	% removal	*G. gelatinosa*	% removal
		Day0	1147±840.342	0%	1147±840.342	0%	1147±840.342	0%
		Day5	1147±840.342	82%	1147±840.342	82%	1147±840.342	81%
	BOD	Day10	212.625±113.171	82%	212±113.39	82%	214±113.088	81%
COFFEE	Mg/L	Day15	212.625±113.171	82%	211.75±113.234	82%	213±113.088	81%
		Day0	684.5±75.238	0%	652.625±74.701	0%	652.625±74.701	0%
		Day5	684.5±75.238	30%	652.625±74.701	32%	652.625±74.701	23%
	BOD	Day10	482.437±82.267	30%	443.75±80.116	32%	502.125±78.178	23%
TEA	Mg/L	Day15	482.437±82.2668	30%	443.75±80.116	32%	502.063±78.181	23%
		Day0	880±20	0%	880±20	0%	880±20	0%
		Day5	880±20	52%	880±20	78%	880±20	38%
	BOD	Day10	420±80	52%	195±5	78%	543±117	38%
SUGAR	Mg/L	Day15	420±80	52%	195±5	78%	543±117	38%

Note: Day 0 represented the control values.

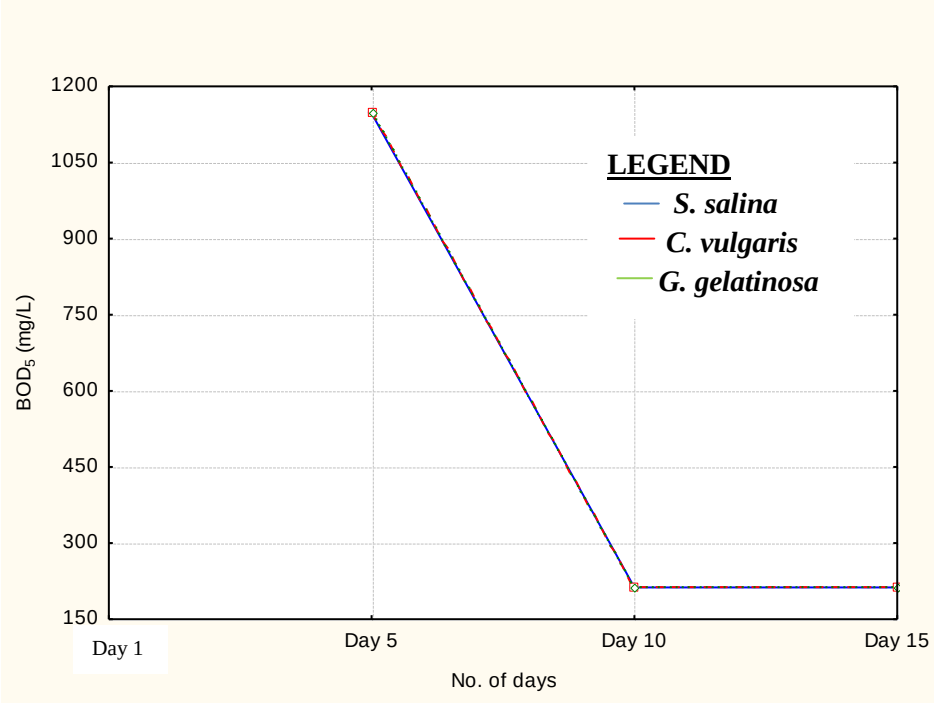


Figure 4. 13 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on BOD of coffee effluent

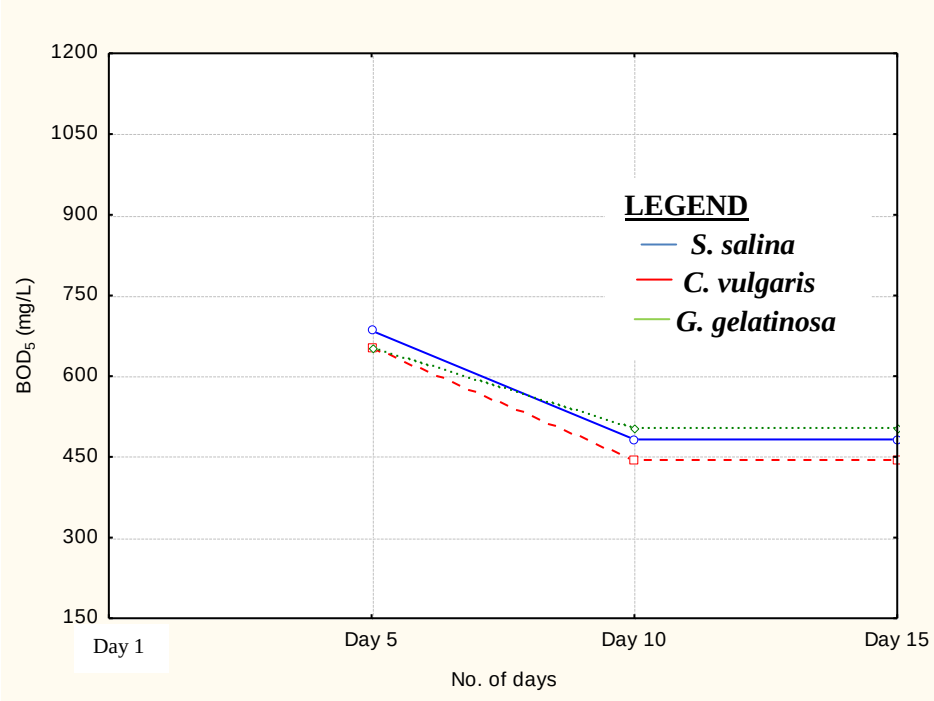


Figure 4.14 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on BOD of tea effluent

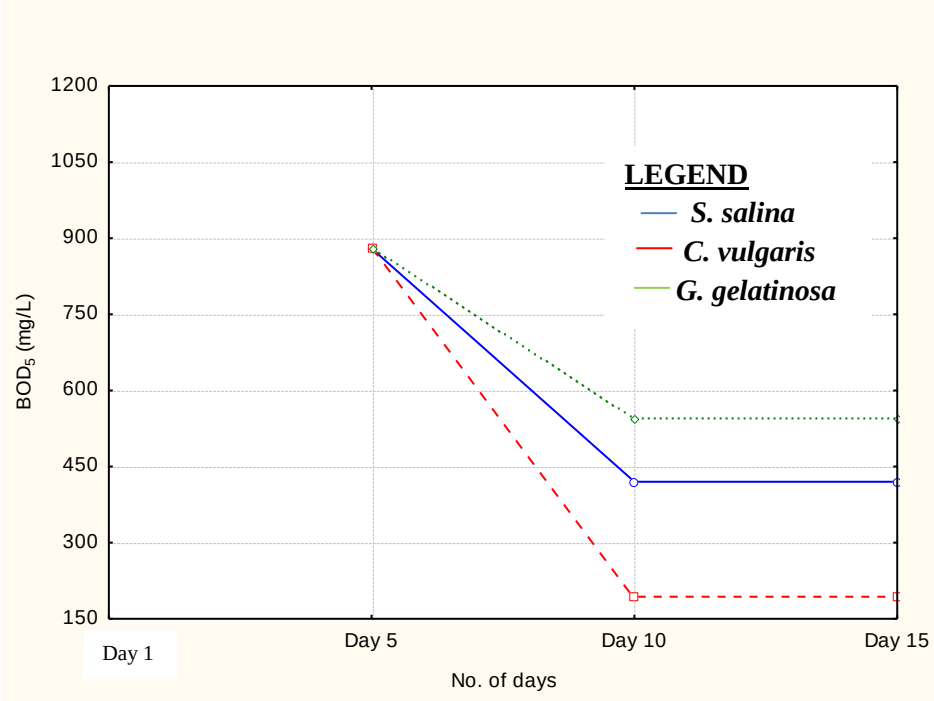


Figure 4.15 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on BOD of sugar effluent.

Phycoremediation efficacy of COD content in the coffee, tea and sugar effluents is shown in Table 4.7 and in Figures 4.16,4.17 and Figure 4.18. However, just like in the previous phycoremediation of BOD as shown in Appendix X there was no significance difference in

the COD reduction between day 1 and day 5 (p-value= 0.61271,p-value= 0.59635 and p-value=619424) for the coffee effluent inoculated with *S.salina,C.vulgaris* and *G.gelatinosa* respectively. Between day 5 and day 10 there was still no significant phycoremediation difference using *C.vulgaris*, *S.salina* and *G.gelatinosa* (p-value=0.946368, p-value=0.938039 and p-value=0.961776) respectively. While between day 10 and day 15 in the coffee effluent *S.salina, C.vulgaris* and *G.gelatinosa* all had no significant difference in phycoremediation efficacy (p-value= 1.0000).

In the phycoremediation efficacy of COD content of tea effluent between day 1 and day 5 there was no significant difference in phycoremediation using *S.salina, C.vulgaris* and *G.gelatinosa* (p-value=0.08066, p-value=0.09392 and p-value=0.26282) respectively. While between day 5 and day 10 the phycoremediation efficacy were also not significantly different (p-value=0.98884, p-value=0.585832 and p-value=0.68542) for *S.salina, C.vulgaris* and *G.gelatinosa* respectively. No significant differences were also noted in the phycoremediation of COD content between day 10 and day 15 in tea effluent with *S.salina* p-value=0.99920, *C.vulgaris* (p-value=0.95712) and *G.gelatinosa* (p-value=0.999141) as shown in reduction trend in Figure 4.17 (see Appendix X).

The phycoremediation of sugar effluent showed that the COD content had no significant difference between day 1 and day 5 (p-value= 0.226044, p-value= 0.081216 and p-value=0.43937) for *S.salina, C.vulgaris* and *G.gelatinosa* respectively. While between day 5 and day 10 *S.salina* and *G.gelatinosa* had no significant difference (p-value=0.12068 and p-value= 0.17529) with *C.vulgaris* having a significant difference in phycoremediation of p-value=0.00784. Between day 10 and day 15 non significant difference of p-value= 1.00000 was recorded for *S.salina, C.vulgaris* and *G.gelatinosa (*see Appendix X).

Table 4.7 Phycoremediation efficacy of *C.vulgaris, S.salina* and *G.gelatinosa* on the COD of coffee, tea and sugar effluent

EFFLUENT TYPE	PARA	DAY	*S. Salina*	% removal	*C. vulgaris*	% removal	*G. gelatinosa*	% removal
	control	Day0	3459±1875.41	0%	3459±1875.41	0%	3459±1875.41	0%
		Day1	3459±1875.41	0%	3459±1875.41	0%	3459±1875.41	0%
		Day5	2331.13±1108.16	33%	2285.75±1081.17	34%	2345.5±1135.38	33%
	COD	Day10	2223.88±1106.56	36%	2165.25±1072.03	37%	2267.25±1132.77	35%
COFFEE	Mg/L	Day15	2223.75±1106.59	36%	2165.25±1071.94	37%	2267.25±1132.77	35%
	control	Day0	1777.81±264.408	0%	1777.81±264.408	0%	1777.813±264.408	0%
		Day1	1777.81±264.408	0%	1777.81±264.408	0%	1777.813±264.408	0%
		Day5	1119.94±250.018	36%	1176.56±225.592	34%	1362.375±250.23	23%
	COD	Day10	1124.63±219.026	37%	1005.06±214.587	44%	1219.375±244.147	31%
TEA	Mg/L	Day15	1124.31±218.956	37%	988.813±209.268	44%	1219±244.173	31%
	control	Day0	4872±1088	0%	4872±1088	0%	4872±1088	0%
		Day1	4872±1088	0%	4872±1088	0%	4872±1088	0%
	COD	Day5	2777.5±533.5	43%	1289±35	73%	3590.5±779.5	26%
	Mg/L	Day10	1250±240	74%	613±49	87%	1724.5±459.5	65%
SUGAR		Day15	1250±240	74%	613±49	87%	1724.5±459.5	65%

Note: Day 0 represented the control values.

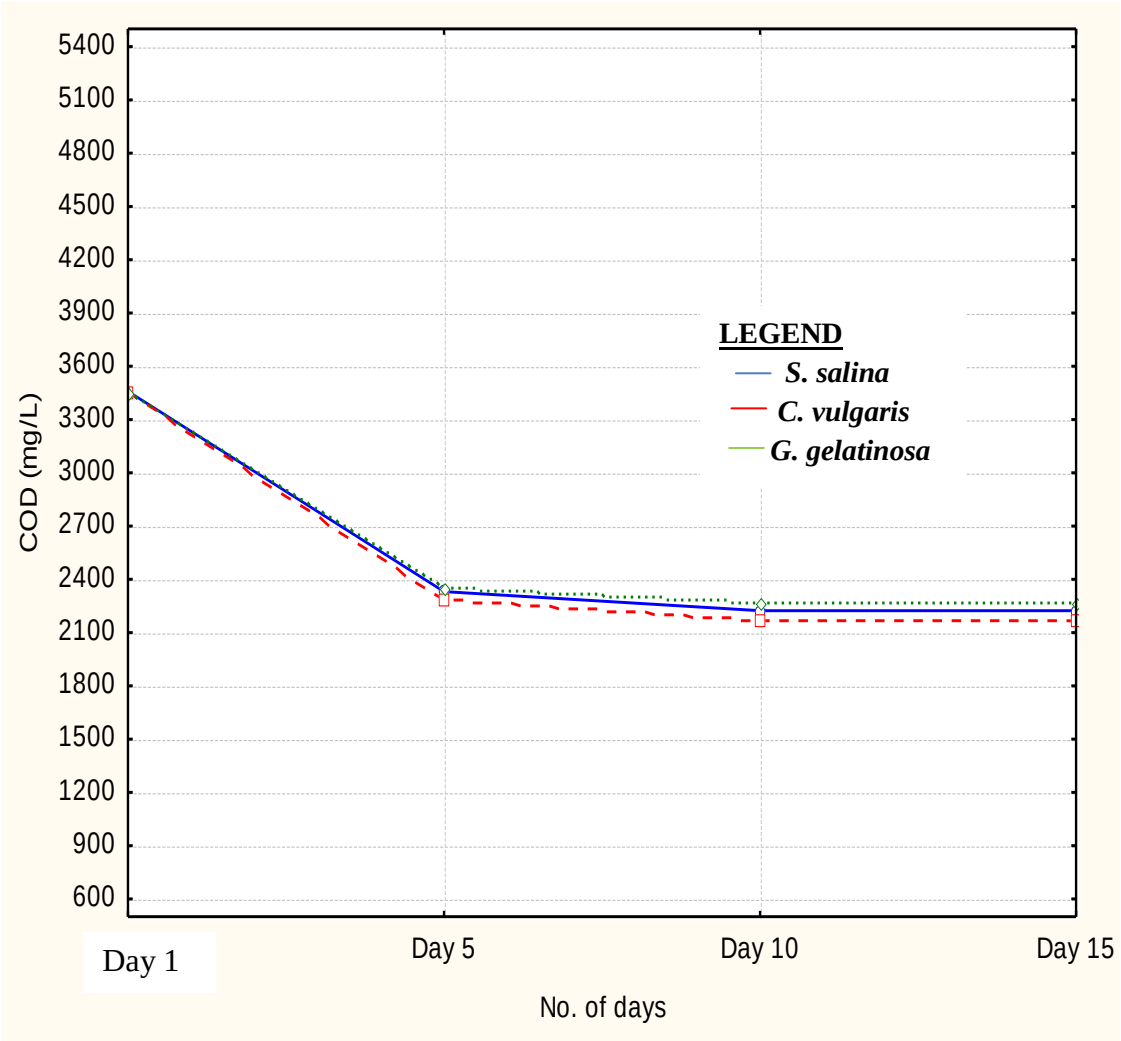


Figure 4.16 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on COD of coffee effluent

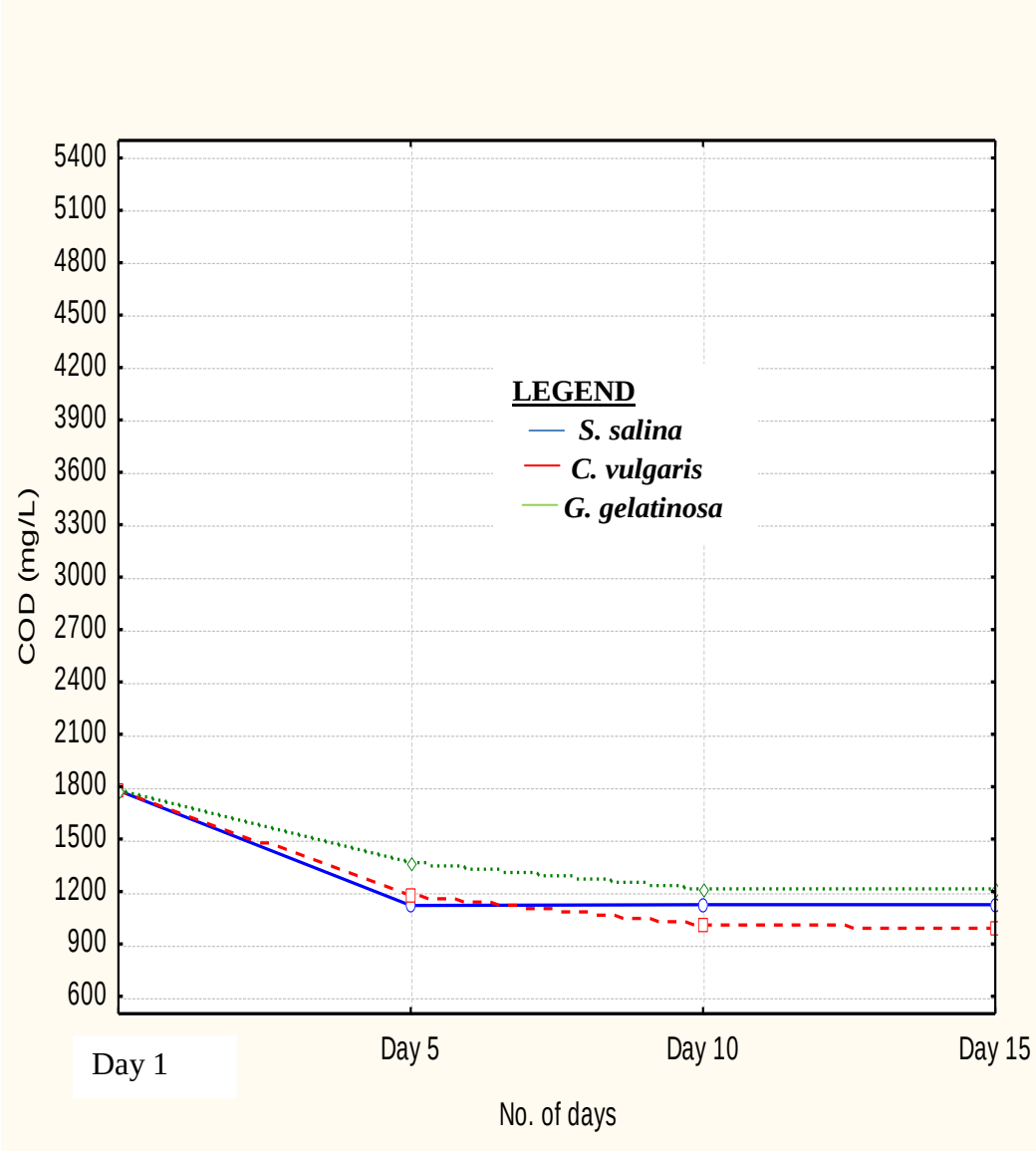


Figure 4.17 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on COD of tea effluent

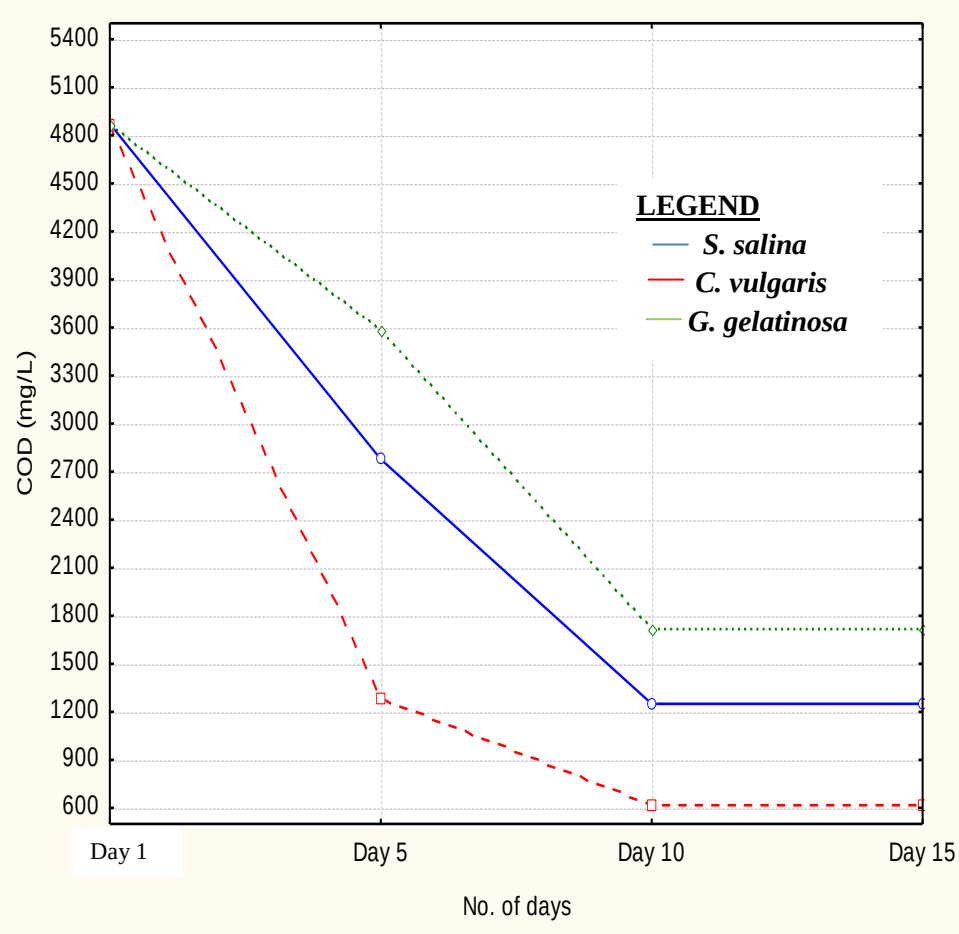


Figure 4.18 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on COD of sugar effluent

Table 4.8 (Figures 4.19, 4.20 and Figure 4.21) shows the effect of the *S.salina*, *C.vulgaris* and *G.gelatinosa* on the conductivity of coffee, tea and sugar effluents.

In coffee effluent the highest Phycoremediation efficacy was noticed between day 1 and day 5 where *C.vulgaris* had a significant difference in Phycoremediation efficacy (p-value=0.03883), while the *S.salina and G.gelatinosa* had no statistical significant phycoremediation differences (p-value=0.11357 and p-value=0.183008) respectively. Between day 5 and day 10 the phycoremediation efficacy were not significantly different with *S.salina* (p-value=0.634313), *C.vulgaris* (p-value=0.653183) and *G.gelatinosa* (p-value=0.693193). While between day 10 and day 15 the phycoremediation efficacies were also not significantly different (p-value=1.00000, p-value=0.999189 and p-value=1.00000) for *S.salina, C .vulgaris* and *G.gelatinosa* respectively (see Appendix X).

However in the phycoremediation efficacy of conductivity in tea effluent using *S.salina* there was a statistical significant difference between day 1 and day 5 (p= 0.000011) and no significant difference between day 5 and day 10 and between day 10 and day 15 (p-value=0.277472 and p-value=1.00000) respectively. The *C.vulgaris* had a significant difference in phycoremediation efficacy between day 1 and day 5 and between day 5 and day 10 while between day 10 and day 15 the phycoremediation was not significantly different (p-value=0.00000, p-value=0.041211 and p-value=1.00000) respectively. While the *G.gelatinosa* had a significant difference in phycoremediation efficacy (p-value=0.000102) between day 0 and day 5 and a non significant difference (p-value=0.452616) between day 5 and day 10 while between day 10 and day 15 no significant difference in phycoremediation was noticed (p-value=1.00000) see Appendix X.

In the phycoremediation efficacy of conductivity in sugar effluent using *S.salina* there was a significant difference (p-value=0.008731 and p-value=0.018309) and a non significant difference (p-value=1.00000) between day 1 and day 5, between day 5 and day 10 and between day 10 and day 15 respectively. The *C.vulgaris* had a significant difference in phycoremediation efficacy (p-value=0.002515) and (p-value=0.008032) between day 1 and day 5 and between day 5 and day 10 while between day 10 and day 15 there was no significant difference in phycoremediation (p-value=1.00000). The *G.gelatinosa* had a significant difference in phycoremediaton efficacy (p-value=0.006557and p-value=0.000152) between day 1 and day 5 and between day 5 and day 10 respectively. While between day 10 and day 15 there were no significant differences in phycoremediation efficacy (p-value=1.00000) (see Appendix X).

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Table 4.8 Phycoremediation effect of *C.vulgaris, S.salina* and *G.gelatinosa* on the conductivity of coffee, tea and sugar effluent;

EFFLUENTTYPE	PARAMETER	DAY	*S. Salina*	% Removal	*C. vulgaris*	% removal	*G. gelatinosa*	% removal
COFFEE	control	Day0	1185.25±378.22	0%	1185.25±378.22	0%	1185.25±378.22	0%
	CONDUCTIVITY ($\mu S/cm^3$)	Day1	1185.25±378.22	0%	1185.25±378.22	0%	1185.25±378.22	0%
		Day5	493.625±157.625	58%	296.625±94.529	75%	592.875±189.063	50%
		Day10	392.25±136.454	67%	238.125±85.43	80%	487.375±181.279	59%
		Day15	392.25±136.454	67%	238±85.411	80%	487.375±181.279	59%
TEA	control	Day0	815.625±95.0978	0%	815.625±95.098	0%	815.625±95.0978	0%
	CONDUCTIVITY ($\mu S/cm^3$)	Day1	815.625±95.0978	0%	815.625±95.098	0%	815.625±95.0978	0%
		Day5	272.875±40.444	66%	188.188±23.071	77%	332.875±50.896	59%
		Day10	212.812±36.234	74%	131.75±12.952	84%	280.5±46.333	66%
		Day15	212.812±36.234	74%	131.75±12.952	84%	280.5±46.333	66%
SUGAR	control	Day0	765±20	0%	765±20	0%	765±20	0%
	CONDUCTIVITY ($\mu S/cm^3$)	Day1	765±20	0%	765±20	0%	765±20	0%
		Day5	460.5±20.5	40%	320±10	58%	518±2	32%
		Day10	285.5±12.5	63%	196±5	74%	315.5±1.5	59%
		Day15	285.5±12.5	63%	196±5	74%	315.5±1.5	59%

Note: Day 0 represented the control values.

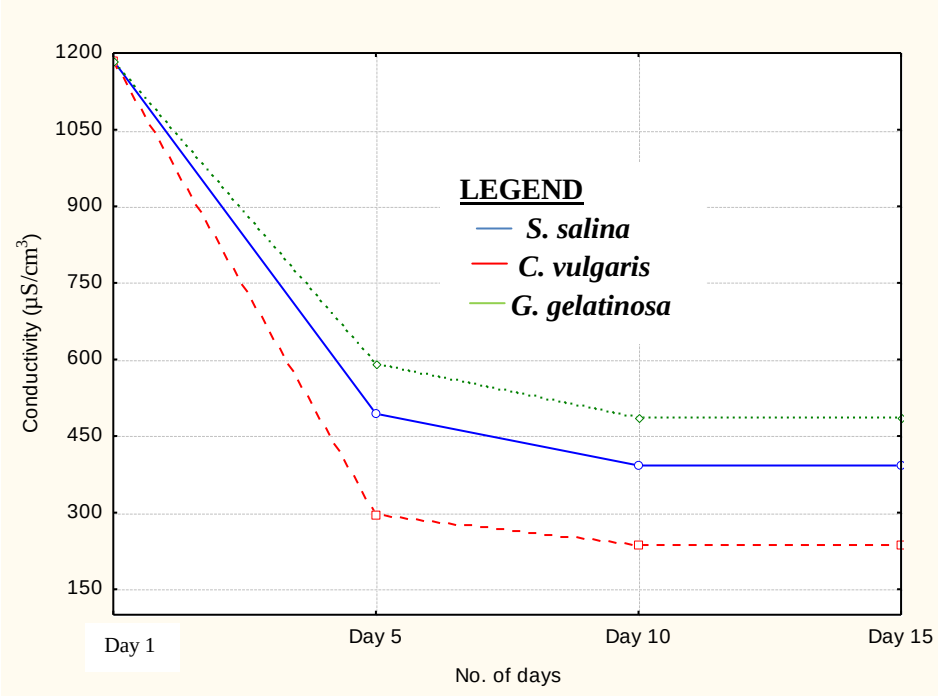

Figure 4.19 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on Conductivity of coffee effluent

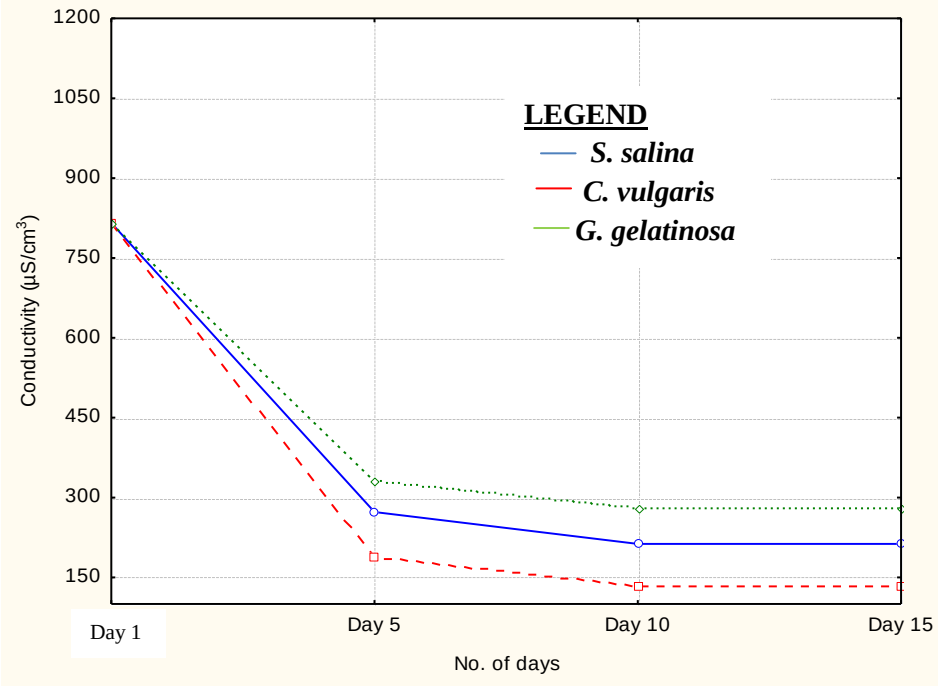

Figure 4.20 Phycoremediation efficacy of *C .vulgaris, S.salina* and *G.gelatinosa* on Conductivity of tea effluent

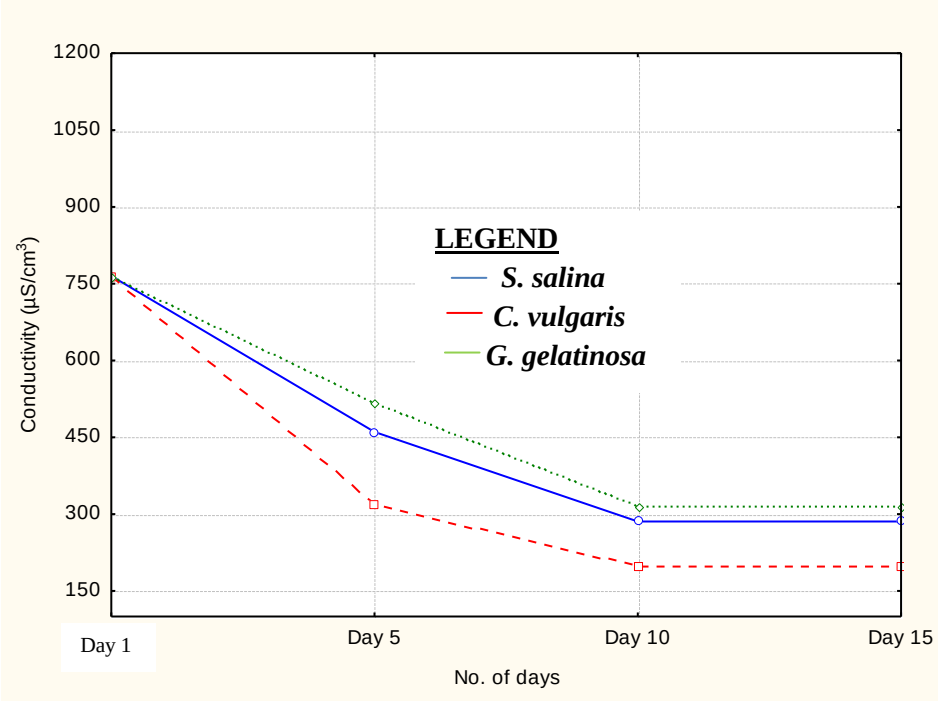

Figure 4.21 Phycoremediation efficacy of *C.vulgaris, S.salina* and *G.gelatinosa* on Conductivity of sugar effluent.

4.1.1 Comparison of the phycoremediation efficacies of *S.salina, C.vulgaris* and *G.gelatinosa* in the coffee, tea and sugar

The comparison of phycoremediation efficacy of *S. salina*, *C.vulgaris* and *G.gelatinosa* in the coffee effluent was determined and found not to vary significantly ($p>0.05$) in all the physicochemical parameters analyzed between and within the groups of the coffee effluent (Table 4.9).

Table 4.9 Comparison of phycoremediation efficacies in coffee effluent (ANOVA)

Paremeter mg/l		Sum of Squares	Df	Mean Square	F	p-value
TDS	Between Groups	88176.333	2	44088.167	.748	0.486
	Within Groups	1238480.625	21	58975.268		
	Total	1326656.958	23			
pH	Between Groups	.986	2	.493	.789	0.467
	Within Groups	13.124	21	.625		
	Total	14.110	23			
Nitrate	Between Groups	26.016	2	13.008	2.393	0.116
	Within Groups	114.164	21	5.436		
	Total	140.180	23			
Phosphate	Between Groups	2.197	2	1.099	.128	0.881
	Within Groups	180.697	21	8.605		
	Total	182.895	23			
BOD	Between Groups	6.583	2	3.292	.000	1.000
	Within Groups	2151429.375	21	102449.018		
	Total	2151435.958	23			
COD	Between Groups	41916.000	2	20958.000	.002	0.998
	Within Groups	2.048E8	21	9751345.833		
	Total	2.048E8	23			
Conductivity	Between Groups	253412.583	2	126706.292	.808	0.459
	Within Groups	3291509.375	21	156738.542		
	Total	3544921.958	23			

In the analysis of tea effluent in Table 4.10, the study results indicated that the comparison of the phycoremediation efficacy of *S.salina, C.vulgaris and G.gelatinosa* were not significantly different ($p>0.05$) in all the physico-chemical parameters of the tea effluent except TDS ($p=0.015$) and conductivity ($p=0.015$) which were significantly different.

Table 4.10 Comparison of phycoremediation efficacies in tea effluent (ANOVA)

Parameter mg/l		Sum of Squares	Df	Mean Square	F	p-value
TDS	Between Groups	68161.625	2	34080.813	4.572	0.015
	Within Groups	335447.375	45	7454.386		
	Total	403609.000	47			
PH	Between Groups	.049	2	.024	.157	0.855
	Within Groups	6.936	45	.154		
	Total	6.985	47			
Nitrate	Between Groups	6.887	2	3.444	3.203	0.050
	Within Groups	48.373	45	1.075		
	Total	55.260	47			
Phosphate	Between Groups	134.458	2	67.229	.402	0.671
	Within Groups	7520.837	45	167.130		
	Total	7655.295	47			
BOD	Between Groups	28171.792	2	14085.896	.137	0.872
	Within Groups	4631727.875	45	102927.286		
	Total	4659899.667	47			
COD	Between Groups	428332.042	2	214166.021	.265	0.768
	Within Groups	36325289.875	45	807228.664		
	Total	36753621.917	47			
Conductivity	Between Groups	177489.542	2	88744.771	4.587	0.015
	Within Groups	870569.438	45	19345.988		
	Total	1048058.979	47			

In the sugar effluent (Table 4.11) the phycoremediation efficacy of *S.salina, C.vulgaris and G.gelatinosa* were found not to vary significantly in most of the physicochemical parameters ($p>0.05$). However, the phycoremediation efficacy of the three species on TDS and conductivity were found to have significant difference with each having a p-value=0.004 (Table 4.11).

Table 4.11 Comparison of phycoremediation efficacy in sugar effluent (ANOVA)

Parameter mg/l		Sum of Squares	Df	Mean Square	F	p-value
TDS	Between Groups	5994.333	2	2997.167	58.197	0.004
	Within Groups	154.500	3	51.500		
	Total	6148.833	5			
PH	Between Groups	.053	2	.027	.457	0.671
	Within Groups	.175	3	.058		
	Total	.228	5			
Nitrate	Between Groups	6.083	2	3.042	4.294	0.132
	Within Groups	2.125	3	.708		
	Total	8.208	5			
Phosphate	Between Groups	1.163	2	.582	1.293	0.394
	Within Groups	1.350	3	.450		
	Total	2.513	5			
BOD	Between Groups	124572.000	2	62286.000	4.645	0.121
	Within Groups	40228.000	3	13409.333		
	Total	164800.000	5			
COD	Between Groups	1244234.333	2	622117.167	3.442	0.167
	Within Groups	542282.500	3	180760.833		
	Total	1786516.833	5			
Conductivity	Between Groups	15460.333	2	7730.167	63.189	0.004
	Within Groups	367.000	3	122.333		
	Total	15827.333	5			

4.2 Phycoremediation efficacy on nitrates and phosphates in the tea, coffee and sugar effluents against WHO permissible stds.

The mean nitrates in the coffee effluents shown in table 4.12 were 5.28±0.6987, 5.50±0.7258 and 7.59±1.0118 after phycoremediation with *S.salina, C.vulgaris* and *G.gelatinosa* for 15 days as shown in Table 4.12. However, the means were lower than the WHO standards (10mg/L) with a significant statistical difference of p= 0.000262, p=0.000445 and p=0.048569 respectively. The mean nitrate for tea effluent were 1.80875±0.2213, 1.43875±0.3152 and 2.36062±2.3606 after phycoremediation in the 15th day and these had a statistical significant difference of p=0.000001, p=0.000001 and p=0.000001 for the *S.salina, C.vulgaris and G.gelatinosa* respectively. The mean values were all found to be significantly

lower than the WHO standards as shown in table 4.12 ($p<0.05$). The mean nitrates for sugar effluent were 4.250000±0.7500, 4.500000±10.00000 and 6.500000±0.500000 and these did not have any significant statistical difference ($p>0.05$) before and after treatment with *S.salina, C.vulgaris* and *G.gelatinosa* up to the 15th day.However the above values were lower than WHO standards.

Table 4.12 Phycoremediation efficacies on nitrates in coffee,tea and sugar effluents against WHO stds

NITRATE	Effluent Source	Species	Mean (mg/L)	WHO STDS	t-value	p-value
	COFFEE	*S.salina*	5.28±0.6987	10	6.7629	0.000262
		C.vulgaris	5.50±0.7258	10	6.20005	0.000445
		G.gelatinosa	7.59±1.0118	10	2.38438	0.048569
	TEA	*S.salina*	1.80875±0.2213	10	37.0106	0.00000
		C.vulgaris	1.43875±0.3152	10	24.2380	0.000000
		G.gelatinosa	2.36062±2.3606	10	24.2380	0.000000
	SUGAR	*S.salina*	4.250000±0.7500	10	7.66667	0.082571
		C.vulgaris	4.500000±10.00000	10	11.0000	0.057716
		G.gelatinosa	6.500000±0.500000	10	7.00000	0.090334

Phosphate values shown in Table 4.13 of the coffee effluents had phosphate means of 2.0388±1.0972, 1.641250±0.8975 and 2.381750±1.1034 with a statistical significant difference of p=0.030682, p=0.007239 and p=0.049401 before and after treatment with *salina, C.vulgaris and G.gelatinosa* respectively. The mean phosphate values for the tea effluent were 30.0500± 3.2475, 28.6125±3.0732 and 32.65625±3.3686 with statistical significance difference of p=0.000001, p=0.000001 and p=0.000001 for *S.salina, C.vulgaris and G.gelatinosa* respectively. Sugar effluent mean phosphate values after phycoremediation in Table 4.13 were 1.50000±0.5000, 1.3500±0.5500 and 2.3500±0.3500 with no significant difference of p=0.090334, p= 0.095213 and p= 0.083598 for *S.salina, C.vulgaris and*

G.gelatinosa respectively. However, after phycoremediation effect the values were below the WHO stds (5mg/L).

Table 4.13 Phycoremediation efficacies on phosphates in coffee, tea and sugar effluents against WHO stds

PHOSPHATE	Effluent Source	Species	Mean (mg/L)	WHO STDS (mg/L)	t-value	p-value
	COFFEE	*S.salina*	2.0388±1.0972	5	2.69904	0.030682
		C.vulgaris	1.641250±0.8975	5	3.74251	0.007239
		G.gelatinosa	2.381750±1.1034	5	2.37282	0.049401
	TEA	*S.salina*	30.0500± 3.2475	5	7.7140	0.000001
		C.vulgaris	28.6125±3.0732	5	7.6835	0.000001
		G.gelatinosa	32.65625±3.3686	5	8.2101	0.000001
	SUGAR	*S.salina*	1.50000±0.5000	5	7.00000	0.090334
		C.vulgaris	1.3500±0.5500	5	6.6364	0.095213
		G.gelatinosa	2.3500±0.3500	5	7.5714	0.083598

CHAPTER FIVE: DISCUSSION

5.1 Phycoremediation efficacy of *C.vulgaris, S.salina* and *G.gelatinosa* on the physicochemical parameters of coffee, tea and sugar effluents

Inorder to protect the public from the possible exposure to pollutants which may have adverse public health effects, the applied phycoremediation technique was able to take up, accumulate and degrade the pollutants found in the tea, coffee and sugar waste water. From the analysis of various physicochemical parameters mainly TDS, COD, BOD, pH, conductivity, nitrate and phosphate it was revealed that *C.vulgaris, S.salina* and *G.gelatinosa* can effectively reduce the pollutants found in coffee,tea and sugar waste water. The physicochemical parameters were therefore measured for 15 days with the initial concentrations of each parameter being recorded in day 0 (zero) and these were used as the controls for each parameter. From the analysed waste water samples the total dissolved solids (TDS) from the tea, coffee and sugar effluents upon phycoremediation with *S.salina, C.vulgaris* and *G.gelatinosa* the concentrations were found to reduce across all the three algal species used as indicated in Table 4.2.

In day 5 the reduction percentages noted were low and this was attributed to the environmental adjustment of the algal species in the mixture within the effluents (Ahmad *et al*., 2013). However as the days progressed especially in day 10 the reduction percentages increased due to the onset of the exponential phase with the stationary phase being witnessed at day 15 as shown in Table 4.2. Kshirsagar, (2014) attributed the lack of phycoremediation activities between day 10 and day 15 to the exhaustion of the TDS in the mixture for the enhancement of the algal activies but from this study it was found that the ligands sites on the algal cell walls were all utilized during ions attachment and exchange and this could have been the real reason for the onset of the stationary phase. Initially the inoculated waste water from tea, coffee and sugar when examined macroscopically as shown in plates 3.10 appeared to have a lot of suspended solids but with time the solids could not be seen since they had been incoperated into dissolved solids thereby increasing the amount of dissolved ions in the waste water through the process of mineralization thus making it easier for the *S.salina, C.vulgaris and G.gelatinosa* to remove them from the waste water as total dissolved solids. The rate of removal of the TDS from the effluents was high in *C.vulgaris* than in *S.salina and G.gelatinosa* and this was because *C.vulgaris* had more functional groups on their cell wall responsible for the high absorption and increased phycoremediation brought about by the various ion exchange potential than *S.salina and G.gelatinosa* and that explains why we had different percentage removal/reduction percentages exhibited by the three algal species as

indicated in Table 4.2 and in Figures 4.1; 4.2 and 4.3. These reductions in TDS were also reported in another similar study by Kshirsagar (2014) who showed varied waste water TDS phycoremediation success with *C. Vulgaris* being more effective than *S. Salina.* However, Kshirsagar (2014) did not establish the reason behind the phycoremediation variability but from the current study it is hypothesised that *C. vulgaris* could be having a lot of functional groups like carboxyl, amino and thiol on its cell wall, and this helped the algae in adsorption and phycoremediation of various ion pollutants of different electrical charges than *S. Salina* with no effect noted on the untreated waste water samples.

Kotteswari *et al*., (2007), Ahmad *et al*., (2013) and Elumalaei *et al*., (2013) using different species of *cyanophyceae* and *chlorophyceae* in effluent phycoremediation also observed an average reduction in TDS of up to 60 %. Rao *et al.*,(2011) and Ahmad *et al.,* (2013) attributed the above reductions in TDS to the phycoremediation efficacy by the algal species by stating that the total suspended solids in the effluents with time were able to dissolve making a strong relationship between conductivity and TDS. Hence it was easy for the *C.vulgaris, S.salina* and *G.gelatinosa* to phycoremediate them from the coffee, tea and sugar effluents.The algal cells adsorps and absorbs the various solids dissolved in the waste water differently due to the variability in the functional groups found on the algal cell walls an aspect which Rao *et al.,* (2011), Ahmad *et al.,* (2013) Kotteswari *et al*., (2007) and Elumalaei *et al*., (2013) failed to consider. However Nanda *et al*., (2010) suggested that the different algal cell wall structures were responsible for the reduced TDS to lower levels, and upon quantification this translated to a high reduction levels because the total suspended solids already present in the effluent may have dissolved, therefore making them to be absorbed and finally taken up by the algal cells and this increased the percentage phycoremediation of the TDS from the waste water by the algal species but Nanda *et al*., (2010) did not subject the algal species to different effluent types inorder to establish the various phycoremediation efficacies an aspect which this study explored in establishing the phycoremediation efficacies.

The electrical conductivity of the tea, coffee and sugar effluents were able to reduce progressively after inoculation with *S.salina, C.vulgaris* and *G.gelatinosa* between day 1(one) and day 5 and between day 10 and day 15. The percentage reduction/ removal efficacy indicated in Table 4.7 showed a similar trend in Phycoremediation efficacy as witnessed in the TDS.The adsorption of the dissolved ions and the conversion of the suspended solids to dissolved substances for the subsequent ion exchange by the algal cells was one of the reason

behind the reduction in the effluents electrical conductivity. Figures 4.19; 4.20 and 4.21 showed the Phycoremediation efficacy of conductivity at which the *S.salina*, *C.vulgaris* and *G.gelatinosa* Phycoremediated the tea, coffee and sugar effluents with all the three species being efficacious in the phycoremediation of electrical conductivity. After the inoculation of the waste waters with the specific algae, the algal species had to adapt first inside the effluent and this was exhibited between day 1(one) and day 5 with the exponential stage seen between day 5 and day 10 and stationary stage setting between day 10 and day 15 and this accounted for the different Phycoremediation efficacy percentages as shown in Table 4.8.

Other studies done by Khemka and Saraf (2015) reported that electrical conductivity of dairy waste water medium highly depended on the availability of dissolved ions notably the bicarbonates, carbon dioxides, hydrogen and hydroxyl ions mainly from the total dissolved solids and the total suspended solids which after dissolving were assimilated by the algal cell thus the reduction in the concentration of the ions from the waste water. Similarly according to Yu *et al*., (2005) phycoremediation was noted to reduce EC value (dSm-1) from inoculation stage all through to the stationary stage (18^{th} day), however in this study the phycoremediation efficacies were noted all through upto 15^{th} day as indicated in Figures 4.19, 4.20 and Figure 4.21 and these findings proved to be more reliable because they included three different types of effluents unlike in the previous studies by Khemka and Saraf (2015) and Yu *et al*., (2005) who considered only one effluent type.

From this study phycoremediation efficacy was found to be due to the ability of algal species to absorb all of the dissolved substances within the effluents through ion exchange and competition for the ligand sites on the surface of the algal cell walls and thereby incooperating the ions to the algal cells with the rate of incoperation and adsorption being depended on species of the algal cells used in the phycoremediation. The *C.vulgaris* was therefore able to remove most of the ions responsible for conductivity more than *S.salina* and *G.gelatinosa* due to the presence of additional functional groups on its cell wall for the attachment of more ions (Nanda *et al*., 2010).

The specific use of micro-algae in the efficient removal of different forms of combined nitrogen and phosphorus has been reported successfully in many studies globally (Shi Jing *et al.,* 2007). In the present study when the polluted coffee,tea and sugar waste water samples were treated with *C.vulgaris, S.salina* and *G.gelatinosa* the nitrates content reduced from 21mg/l to 5.5mg/l while *G.gelatinosa* reduced from 21mg/l to 7.5mg/l. Similar reductions

were noted in the tea waste water however the highest phycoremediation efficacy was noted in sugar effluents and these reductions efficacies as noted, however changed with the inoculated algal species as shown in Table 4.4 and Figures 4.7,4.8 and 4.9 since phycoremediation efficacy is species depended, (Kshirsagar., 2013). In the Phycoremediation of the phosphates content in the three effluents the *C.vulgaris, S.salina* and *G.gelatinosa* also showed varied phycoremediation efficacies though the tea effluent had the highest phosphates content with an initial concentration of 69.2mg/l which was reduced to 30mg/l and this was phycoremediated successfully as indicated in Figures 4.10,4.11 and 4.12 whereby the reduction trend in the 15 days period of incubation just as in the case of nitrate reduction were attributed to the nutrient absorption during the ion exchange process which lead to the algal growth with the nitrates and phosphates mainly being derived from the mineralization process.

The gradual reduction of the phosphorous and nitrates from the waste water was also attributed to the fact that nutrients had been absorbed from the waste water by the *C.vulgaris, S.salina and G.gelatinosa,* mainly for their growth. Sivasubramanian *et al.,* (2012) noted that phosphorous and nitrate concentrations in the waste water mediums were related to the growth of the micro-algae and the eventual reduction in the waste water without establishing their origin. However from this study mineralization was found to be one of the major factor in the increase of nitrates and phosphates in the waste water as observed overtime when some organic matters managed to dissolve thus accounting for the increased inorganics like the nitrates and phosphates. Additionally Phosphorous and nitrates concentrations are often limiting nutrients in the *C.vulgaris, S.salina* and *G.gelatinosa* growth and the cells can easily assimilate and store these nutrients diminishing their concencentrations in the coffee, tea and sugar waste water (Sivasubramanian *et al*., 2009).

Phycoremediation of nitrates from industrial effluents by Kshirsagar (2013; 2014) showed nitrate reductions using *C. vulgaris* of 70%, 60%, 93.43% and 89.84 %, for days 1,5,10 and 15, corresponding to 40%, 53.33%, 93.38% and 86.53 % by *S. salina* respectively, however these results were only restricted to one type of effluent making the the results not to be more reliable unlike in this study were three effluents namely coffe, tea and sugar were considered. Dominic *et al., (*2009) also established that nitrate content reduction rate was high at 96.23% using *Synechocystis salina* with the values having been reduced from 5.3µmol/l to 0.2µmol/l but too did not subject the *Synechocystis salina* to the different types of effluents inorder to a

certain an effective phycoremediation efficacy. Yoshida *et al*., (2006), Sreesai and Pakpain (2007) also showed that the nitrate content of the effluent samples inoculated with *Synechocystis salina* reduced significantly with the time of incubation where they noted that the initial value of nitrate was 64.0 mg/L and decreased to 14.2 mg/L. The maximum reduction occurred on day 12 and onwards which was recorded at 77.8% while the phycoremediation efficacy with *C.vulgaris* occurred on day 8 which was 88%.Whereas Tam and Wong (1990) reported a phycoremediation efficacy of 88% of nitrates on effluent treated with *G.gelatinosa.*

Similary phycoremediation efficacy studies by Dominic *et al., (*2009) using *Synechocystis salina* showed that phosphates content in effluent samples reduced by 64.52%, while Gonzalez *et al.*, (1997) studying industrial waste water found that the microalgae *Chlorella vulgaris* was able to remove 50% of total phosphates in effluent samples. However Weerawattanaphong, (1998) reported a maximum total phosphates reduction of 77% after 8 days of phycoremediation.Mamun *et al., (*2012), in a study on industrial waste water treatment concluded that the peak of *Chlorella vulgaris* growth on the fifth day of the batch culture the total phosphates removal efficacy was 40.7%. Other studies by Kumar and Saramma, (2012), Tam and Wong (1990) also established phycoremediation efficacies of 88.82% and 90% reduction in the phosphate content on the 15th day of *G.gelatinosa* cultivation.

However, unlike in the above cited studies, this study which was done under laboratory conditions was able to establish that the maximum phycoremediation efficacies of *C.vulgaris, S.salina* and *G.gelatinosa* occurred at day 10 in the three effluents thus making the current results to be more reliable since they involved more than one algal species and also more than one effluent type unlike in the above cited studies which focused only on one effluent type and one algal species without considering phycoremediation variabilities manifested within different algal species.

The BOD and the COD concentrations in the tea ,coffee and sugar effluents showed the various levels of toxicities within the effluents and the amounts of oxygen needed by *S.salina, C.vulgaris* and *G.gelatinosa* in order to breakdown the organic matter found in the waste water samples. From the study, coffee effluent had the highest demand for oxygen because of the high organic matter inform of coffee husks with an initial BOD of 1147 mg/l unlike tea and sugar which had BOD of 685mg/l and 880mg/l respectively as indicated in

Table 4.6. This meant that the coffee waste water had more organic and inorganic pollutants than tea and sugar waste water thus requiring more oxygen molecules to break them down therefore releasing energy for the growth to the algal species which inturn will drive the process of photosynyhesis.

$$6CO_2 + 6H_2O \rightleftharpoons C_6H_{12}O_6 + 6O_2$$

Carbon-dioxide Water Carbohydrate oxygen

Six molecules of CO_2 were consumed to release six molecules of oxygen which in effect reduces the BOD.

However it was also observed that upon phycoremediation facilitated by the atmospheric carbon dioxide more oxygen molecules were added into the effluents through the process of photosynthesis by *S.salina, C.vulgaris* and *G.gelatinosa* algal species thereby reducing the oxygen demand in these effluents as reported by Elumalaei *et al*. 2013.

Figures 4.13.4.14 and figure 4.15 showed the progressive reduction in the oxygen demand in the coffee, tea and sugar effluents respectively. Differences in phycoremediation efficacies were also noted with *C.vulgaris* showing a better phycoremediation efficacy than *S.salina* and *G.gelatinosa* in all the three effluents as shown in Table 4.6. However these changes in Phycoremedation among the algal species could be attributed to the different functional groups found within the algal species and which are important in the bioabsorption of various waste water pollutants through ion exchange mechanism (Elumalaei *et al*. 2013).

There was a progressive reduction in COD noted between day 5 and day 10 and this was due to the onset of the logarithmic growth phase of the cells of *S.salina, C.vulgaris* and *G.gelatinosa* with the high algal photosynthetic activities being the other additional reason for the reductions as depicted in Table 4.7 and summarized in Figures 4.16; 4.17 and Figures 4.18.

The current study therefore showed a marked decline of initial COD and BOD values from high levels to lower levels at day 15. This indicated that if the concentrations of the algal inoculums can be checked aganist the initial COD and BOD concentrations in a waste water sample at day 1, then desired COD and BOD values can be realized in all the algal species used irrespective of the waste water under investigation as shown in the phycoremediation efficacies in Figure 4.13 upto figure 4.18. phycoremediation study by Sharma and Shakeel

(2013) showed an increasing reduction in both COD and BOD levels and this was observed to relate well with the current study though they only subjected one effluent type to one algal species and also did not give the reasons for progressive reduction in COD and BOD. The progressive reduction in COD and BOD as indicated earlier was therefore due to high photosynthetic activities and increased algal growth rate. High oxidations of carbons releasing carbon dioxide was also responsible for the reduction of COD and BOD values, and similarly the enhanced biological conversion of the waste water organic matter and the increased biodegradation of the same due to algae might have been the other additional reason (Elumalaei *et al*. 2013).

The phycoremediation effect of *C.vulgaris, S.salina* and *G.gelatinosa* on the pH of the three effluents was reported to increase between day 1 and day 5 and then decrease in day 10 in all the effluents before stabilizing between day 10 and day 15 where no further increase or decrease was recorded.The phycoremediation efficacy was significantly different ($p<0.05$) in all the days of remediation across all the species, however the effect of *S.salina* and *C.vulgaris* on the coffee effluent was not statistically significant ($p=1.0000$). From the current phycoremediation findings on the pH of the coffee, tea and sugar effluents, the study established a progressive increase in pH from neutral to alkaline across all the different effluents. During the phycoremediation process, while the other physicochemical parameters were decreasing the pH levels showed an increase at first before remaining at a mean of 8.0 across all the three algal species used in the treatments. The reason for the rise in pH levels was attributed to the reduction in the dissolved CO_2 concentrations through photosynthesis which, in turn, raised the pH level (Rao *et al*., 2011).

However, Arbib *et al.*,(2011) and Zhao *et al.*,(2016) showed that the pH increases during the growth stages of microalgae and this was due to a shift in the chemical equilibrium system among carbon dioxide, carbonic, carbonate and hydroxide. Whereby, CO_2 first combined with H_2O to form H_2CO_3 which dissociated into HCO_3^- and H^+. Then, the carbonic dissociated in carbonate. During the growth, the amount of carbonate increased and carbonic decreased, resulting in the increase of hydroxide and thus the increase in value of pH. Khemka and Saraf (2015) however noted that the electrical conductivity shows irregular behaviours during remediation thus cannot be attributed to the rise in pH as suggested by Zhao *et al.*,(2016), while Yu *et al.*, 2005 also noted that phycoremediation reduces EC value (dSm-1) due to the absorption of the nutrients in the waste water medium. A similar study by

Borowitzka, (1998) and Rao *et al*., (2011) established that the inorganic species used mainly by microalgae are usually CO_2 and bicarbonate, the latter requiring the enzyme carbonic anhydrase to convert it to CO_2 for use in photosynthesis process.

The electrical conductivity irregular or regular behaviours noted by Khemka and Saraf (2015) and Zhao *et al.*,(2016) could not be proved with only one effluent type however in this study three effluents were considered and the pH of all the three effluents were found to increase towards alkalinity from day 1 to day 5 and then start decreasing between day 5 and 10 before stabilizing at day 15 thus establishing the regular behavior of the pH though under the laboratory conditions only.

5.1.1 Comparison of the phycoremediation efficacies of *S. salina, C.vulgaris* and *G.gelatinosa* in tea, sugar and coffee effluents.

The phycoremediation efficacy of *S. salina, C.vulgaris* and *G.gelatinosa* in the coffee effluent was determined and the comparison of phycoremediation efficacy between and within groups was found not to vary significantly in all the physicochemical parameters analyzed due to the structural cell wall similarities of *C.vulgaris, S. salina,* and *G.gelatinosa* (Lesmanaa *et al*., 2009). The comparison of phycoremediation effect of the tea effluent showed that the phycoremediation efficacy of *S.salina, C.vulgaris and G.gelatinosa* did not also vary significantly ($p>0.05$) in all the physico-chemical parameters, except TDS and conductivity as shown in Table 4.10.

However there was a significant difference in the comparison of phycoremediation efficacy of the effluent conductivity across all algal species in the tea effluent ($p<0.05$), while the comparison of pH , phosphate, nitrates, BOD and COD between and within groups showed that phycoremediation efficacy did not vary significantly across all the algal species ($p>0.05$) and this Lesmanaa *et al*., (2009) attributed on the components of the algal cells, especially through cell surface ligand sites and the spartial structure of the cell wall.

However this study established that various polysaccharides, proteins and lipids existing in algal cell walls of *S.salina, C.vulgaris* and *G.gelatinosa* may have played a critical role in binding of the ions dissolved in the waste water whereas the functional groups mainly carboxyl and amino found on the cell walls may have potentiated the binding and the exchange of the ions with the electromotive force being the key driver in the ion exchange

thus increased biosorption of the ions leading to a high phycoremediation efficacy of the TDS in tea, coffee and sugar waste water.

Therefore the highest phycoremediation efficacy in tea, coffee and sugar waste waters was witnessed at high pH while the lowest phycoremediation efficacy was witnessed at low pH. Increase in pH of the waste water therefore increased the biosorption potential of the algal species as seen in Figures 4.4; 4.5 and 4.6 on pH and when this increase in pH was compared with the other phycoremediation efficacy e.g Figures 4.7 upto Figure 4.21 the increase in biosorption and phycoremediation efficacies of the pollutants was evident between the three algal species and within the tea, coffee and sugar waste water. Protonation and deprotonation of the *S.salina, C.vulgaris* and *G.gelatinosa* functional groups was therefore controlled by the pH of the waste water medium which affected the biosorption and phycoremediation capacities. Therefore at low pH the carboxylic groups being acid and common in *S.salina, C.vulgaris* and *G.gelatinosa* existed in the protonated state due to the presence of excess H^+ and H_3O^+ therefore, repulsive forces of these protonated groups with positively charged pollutant ions could have been responsible for the lower biosorption capacities in the waste waters at low pH as suggested by Rao *et al.,* (2011) an aspect which Lesmanaa *et al*., (2009), Sivasubramanian *et al*., (2009) and Kshirsagar (2014) failed to establish in their phycoremediation studies.

In the sugar effluent the comparison of the phycoremediation efficacy of *S.salina, C.vulgaris and G.gelatinosa* were found not to vary significantly across all the physicochemical parameters ($p>0.05$). However the phycoremediation efficacy of *S.salina, C.vulgaris* and *G.gelatinosa* on TDS and conductivity were found to have a significant difference as shown in Table 4.11.This implied that the phycoremediation efficacies of *S.salina, C.vulgaris* and *G.gelatinosa* were almost the same and that the three species were all efficacious in the phycoremediation of coffee,tea and sugar effluent pollutants with conductivity values being affected by the amount of total solids dissolving and that's why TDS and conductivity where all found to have significant differences in phycoremediation efficacies unlike in the other parameters. Therefore the phycoremediation efficacy exhibited by *C.vulgaris, S.salina* and *G.gelatinosa* can be attributed to their biosorbent and adsorption properties which originate from their porous cell walls, allowing the free passage of methane, nitrates and phosphates in an aqueous solution. Moreover, their cell wall's constituents provided an array of ligands

with different functional groups like amino, carboxyl and thiol capable of binding various pollutants (Lesmanaa *et al*., 2009).

The current phycoremediation study compared relatively well with other related studies by Sahu (2014), Murali and Nisha (2009) and Dominic *et al., (*2009) on the various waste water studies on physicochemical parameters which showed a maximum reduction of COD, phosphates, nitrates and BOD after 21 days, however the maximum phycoremediation efficacies in this study was observed after 15 days and this was because it involved three effluents and three algal species of different phycoremediation variabilities unlike in the above cited studies which only employed one effluent type thus this study results proved to be much more reliable than the above previously cited. Therefore microalgae *Chlorella vulgaris*, *Synechocystis salina* and *Gloeocapsa gelatinosa* were all able to improve water quality by removal of nitrates and phosphates with their results showing that all the three algae were highly efficacious in pollutant reductions of the waste waters. From the current results however the *C.vulgaris* species was more efficacious in uptake of nitrates relative to *S.salina* spp and *G.gelatinosa* spp. The decreasing order of nitrate and phosphate reduction efficiency was *Chlorella vulgaris> Synechocystis salina >Gloeocapsa gelatinosa*. All the three algae showed high efficiency in the reduction and removal of nutrients.

5.2 Phycoremediation efficacy of *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa* on nitrates and phosphates in tea, coffee and sugar effluents against WHO permissible stds

The phycoremediation effect of nitrates in coffee effluent were assessed effectively against the WHO standards (10mg/l) whereby a significant difference in phycoremediation efficacy was reported for the case of *S.salina, C.vulgaris* and *G.gelatinosa* as shown in Table 4.12.The coffee effluent was found to have the highest levels of nitrate concentrations in individual factory effluents though when subjected to phycoremediation and assessed against the WHO standards (10mg/l) the levels reduced to below 10mg/l. The nitrates levels were attributed to mineralization process and the nitrate fertilizers commonly applied in the coffee farming while the different phycoremediations were due to the assimilation of nitrogen compounds by the *S.salina, C.vulgaris* and *G.gelatinosa* during the logarithmic phase of their growth (Sahu, 2014).

The same trend was noted in sugar effluents which had also a high nitrate content than the tea effluent suggesting that sugar cane farming relied more on the nitrate fertilizers.The coffee

husks and the sugar cane plant remnants being organic in nature could have been converted into inorganic compounds through mineralization process therefore increasing the concentration of the nitrate inorganic compounds. The assessments of the phycoremediation efficacies of *S.salina, C.vulgaris* and *G.gelatinosa* on the nitrate content of the sugar effluent against the WHO standards (10mg/l) were therefore found to have insignificant differences as shown in table 4.12. Most of the nitrates as suggested by Akpor and Muchie (2010) might have been taken away from the farms through runoffs while the remaining nitrate nutrients may have been fully assimilated during the growth of the sugarcane thus accounting for the remaining mean nitrate levels in the effluents to be assessed against the accepted WHO value of 10mg/l through phycoremediation efficacies.

However excess nitrates may damage the photosynthesis organs thus decreasing the photochemical efficiency of *C.vulgaris, S.salina* and *G.gelatinosa* used in the phycoremediation Akpor and Muchie (2010) but because the mean values of the nitrates from most of the study areas did not highly exceed the permissible WHO standards, therefore it meant that phycoremediation efficacy was able to proceed without any challenge. The various phycoremediation efficacies assessed against the WHO standards of 10mg/l nitrates were therefore shown in Table 4.12.The mean nitrate in tea effluent was phycoremediated successfully with a statistical significant difference ($p=0.000001$, $p=0.000001$ and $p=0.000001$) for the *S.salina, C.vulgaris* and *G.gelatinosa* respectively.This was because tea farming in the studied area rarely used nitrate fertilizers but instead used the phosphate fertilizers as exhibited in the effluent values shown in Table 4.13. The mean nitrate values were all found to be significantly lower than the WHO stds ($p<0.05$).

From the study, the phycoremediation of phosphates in coffee effluent was significantly different and so were the phosphates in sugar effluent for *S.salina, C.vulgaris and G.gelatinosa.* However the mean phosphate values for the tea effluent showed a statistical significant difference for *S.salina, C.vulgaris and G.gelatinosa* as indicated in Table 4.13.This however showed that phycoremediation of phosphates was highly efficacious in tea effluent whereby the initial phosphate values where high above the allowable WHO limits of 5mg/l and upon assessment against the WHO (5mg/l) the phosphate phycoremediation showed a great significant difference of $p= 0.00001$ than in any other effluent. However after all the phycoremediation effects the values were found to be below the WHO stds (5mg/l) and in a related study Akpor and Muchie (2010) found out that when phosphates are in high

levels they tend to get accumulated, taken up stored and assimilated by the algal cells very fast since phosphate is a limiting nutrient and that explains why the effluents with the highest amounts of phosphates had a phycoremediation significant statistical difference of p= 0.00001.

From this phycoremediation efficacy of *S.salina, C.vulgaris* and *G.gelatinosa* on the nitrates and phosphates in coffee, tea and sugar effluents against the WHO standards it was also established that when the pH of the algal and effluent mixture were high the phycoremediation of the nitrates and phosphate was also high and the reverse was true as seen in figures 4.3 upto 4.12. In another study on bioremediation of polluted waste water influent, Akpor and Muchie (2010), Murali and Nisha (2009) established that presence of ions affected the phycoremediation efficacy of algal species due to the variability in the ions charge and that of the ligand sites.

Akali *et al*., (2011) also agreed with the current study and established that phosphates in water can stimulate the growth of planktons and algae which provides food for the fish. However, if an excess of phosphates enters the waterway i.e. over 5mg/l (eutrophication or over-fertilization), algae and aquatic plants will grow wildly, choke up the waterway and use up large amounts of oxygen. This rapid growth of aquatic vegetation eventually dies and as it decays it uses up oxygen. This process in turn causes the death of aquatic life because of the lowered dissolved oxygen levels (Akali *et al*., (2011). High phosphates are toxic to both human and animals and can cause digestive problems (Olguin, 2003) while nitrogen compounds in waste water are toxics of non-ionized ammonia to fish and other aquatic organisms, and interferes with disinfection where a free chlorine residual is required and methemoglobinemia condition due to excess nitrate concentrations (above 10mg/l) in drinking water (Olguin, 2003; Akali *et al*., 2011; Khazenzi *et al., 2013*).

The current study was found to be similar with other previous studies. Abioye *et al.,* (2014) on treatment of textile waste water using *Candida zeylanoides and Saccharomyces cerevisiae* incubated for 15 days showed that *S.cerevisiae* effectively assesed the nutrients pollutants against the WHO with a mean of 66% reduction in nitrate and phosphate levels while *C. zeylanoides* had 57.3% reduction efficiency in nitrates and phosphates, while a consortium of the two species showed lower remediation potential of 36.9% for the combined nutrients. In the current study however, incubation was done for 15 days and the phycoremediation efficacies were recorded after every 5 days thus it was possible to monitor the

phycoremediation efficacies which was not the case in the above cited study. Phosphates and nitrates removal efficacy from algal inoculated dairy waste water studied by Rajasri and Goutham (2013) showed also a similar relationship with the present study where a significant decrease in nitrogen and phosphorous levels due to algal uptake were noted with a removal efficacy of 97% and 96% respectively. However, only one algal species was used unlike in this study were three algal species and three effluents were used in the phycoremediation study. Other waste water phycoremediation studies by Kshirsagar (2013; 2014), Rao *et al.,* (2011) showed nitrate reductions using *C. vulgaris* of 70%, 60%, 93.43% and 89.84 %, respectively, corresponding to 40%, 53.33%, 93.38% and 86.53 % by *C. salina* and these results were also found not to be reliable because they subjected the phycoremediation process to only one effluent type and the incubation periods were not properly specified unlike in this study were the incubation period and the recording of the results were specified and three effluents used in the phycoremediation process..

CHAPTER SIX: SUMMARY OF FINDINGS, CONCLUSION AND RECOMMENDATION

6.1: Summary of findings;

The section summarizes the main research findings which if taken into consideration will help in eradicating most of the public health problems associated with contaminated waste water. The research hypothesis which were sought were: There was no relationship between the phycoremediation efficacy of *Chlorella vulgaris, Synechocystis salina and Gloeocapsa gelatinosa* on the physicochemical parameters of coffee, tea and sugar effluents from Bungoma, Nandi and Kakamega. There was also no relationship in the assessment of phycoremediation efficacy of *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa* on the nitrates and phosphates in coffee, tea and sugar effluents against the WHO standards.

From the study it was found that:

1. There was a relationship in the phycoremediation efficacy of *Chlorella vulgaris,Synechocystis salina* and *Gloeocapsa gelatinosa* on the physicochemical parameters of the coffee,tea and sugar effluents with a statistical insignificant variation difference of $p>0.05$. The three algal species were found to be efficacious in reducing the BOD,COD,TDS,conductivity levels within the effluent which in turn helped in bioremediating the effluent making it suitable for discharge.The relationship in the phycoremediation efficacy was due to the presence of the cell wall constituents within *Chlorella vulgaris,Synechocystis salina* and *Gloeocapsa gelatinosa* which have an array of ligands with different functional groups capable of binding various pollutants within the effluents (Lesmanaa *et a.l,* 2009). However the pH of the effluents initially increased between day1 and day 5 before decreasing between day 5 and day 10 and then stabilizing between day 10 and day 15.This trend in phycoremediation was found to be related in *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa*. Rao *et al.,* (2011) attributed the initial rise in pH to the reduction in dissolved CO_2 concentrations through photosynthesis process.
2. There was also a relationship between the assessment of phycoremediation efficacy of *Chlorella vulgaris, Synechocystis salina* and *Gloeocapsa gelatinosa* on the nitrates and phosphates in coffee, tea and sugar effluents against the WHO standards (10mg/L and 5mg/L). In all the effluents the nitrates and phosphate levels were reduced to almost the WHO allowable limits. Lesmanaa *et al.,* (2009) found out that nitrates and phosphates were required for the growth of the *Chlorella vulgaris, Synechocystis salina* and

Gloeocapsa gelatinosa therefore their assimilation into the algal cells was almost related with the assessment of their phycoremediation efficacy against the WHO standards which depended on the cell wall constituents of the individual algal species.

6.2 Conclusion

The phycoremediation efficacy of *S.salina, C.vulgaris* and *G.gelatinosa* on the physiochemical parameters of public health importance varied significantly between coffee, tea and sugar waste waters ($p<0.05$). The sludge formation decreased with increased decline in TDS levels.The comparison of *S.salina, C.vulgaris* and *G.gelatinosa* efficacy in the phycoremediation of the coffee, tea and sugar effluents were all found to be effective in removal of xenobiotics which could have had adverse public health effects with a statistical insignificant variation difference ($p>0.05$) reported across all the species.The phycoremediation efficacies of the three algal species were found to be in the order,*C.vulgaris> S.salina> G.gleocapsa* across all the three effluents studied. The assessment of phycoremediation efficacy of *S.salina, C.vulgaris* and *G.gelatinosa* on nitrates and phosphorous in coffee, tea and sugar effluents against the WHO standards showed a reduction trend in the levels of the physicochemical parameters in all the effluents to near acceptable WHO standards of 10mg/l and 5mg/l for nitrates and phosphates respectively.However not all the WHO standards could be attained due to the differences in pollutant concentrations in the three types of effluents. Phycoremediation process was therefore effective upto day 10, with day 15 registering on change in phycoremediation efficacy. Nitrates were significantly the highest nutrient pollutants in coffee and sugar waste waters posing a possible likelihood of a public health problem known as methaemoglobin to the community while phosphates were found to be significantly high in tea waste water and this too could pose a public health problem.

6.3 Recommendations

The study therefore suggests the following recommendations;

- *S.salina, C.vulgaris* and *G.gelatinosa* should be adopted for use in the phycoremediation of physico-chemical parameters in coffee, tea and sugar waste water in order to reduce the possible waterborne disease outbreaks.
- *S.salina, C.vulgaris* and *G.gelatinosa* should be used in the phycoremediation of nitrates and phosphates pollutants of public health effects in coffee, tea and sugar waste water based on the WHO standards.
- Advocacy on the use of phycoremediation in the removal of pollutants in waste water.
- Trainings and capacity building on phycoremediation technologies should be enhanced in the studied areas so as to mitigate on the possible waterborne diseases.
- Treatment ponds in all the factories needs to be constructed and maintained especially in areas were effluent is discharged directly into the rivers like in coffee factories and in sugar factories so as to allow for *S.salina, C.vulgaris* and *G.gelatinosa* inoculation and propagation.

6.4 Recommendation for further studies

A further study on the effect of phycoremediation efficacy in reduction of waterborne diseases associated with polluted coffee, tea and sugar waste water needs to be done inorder to acertain their public health effect.

REFERENCES

Aarti, N. Sumathi, P. Subrahmanian, V (2008) Phycoremediation to improve algal waterquality. *Indian Hydrobiology*.**11** (1)**:**173–184.

Abdel-Raouf, N. Al-Homaidan, A.A. Ibraheem, I.B.M. (2012) Microalgae and waste water treatment. *Saudi Journal of Biological Sciences*.**19 (**3)**:**257–275.

Abioye, O. P. (2011).Biological remediation of hydrocarbon and heavy metals contaminated soil. *Malaysian Journal of Science*.**28:1**-17.

Abioye, O. P. Mustapha, O.T. and Aransiola, S.A.(2014). Biological Treatment of Textile Effluent Using *Candida zeylanoides* and *Saccharomyces cerevisiae* Isolated from Soil.Intech, Rijieka. 2011.

Agale, M.C. Patel, N.G. and Patil, A.G. (2013).Impact of sugar industry effluents on the quality of ground water from Dahiwad Village. *Archives of Applied Science Research*. ***5***(3)**:**58-60.

Ahmad, F. Khan, A.U. Yasar, A. (2013). Comparative phycoremediation of sewage water by various species of algae. *Pakistan Academic Science Journal*.***50***(2)**:**131–139.

Akali, N.M.Nyongesa, N.D.Neyole, E.M. and Miima, J.B.(2011). Effluent discharge by Mumias Sugar Company in Kenya: An Empirical Investigation of the pollution of River Nzoia. *Sacha Journal of environmental studies***.1**(1)**:**1-30.

Akpor OB, Muchie M. Bioremediation of polluted waste water influent (2010). Phosphorus and nitrogen removal.*Scince Resource. Essays Journal*.**5** (1)**:**3222-3230.

Anandaraj,V. Subrahmaniyan, V.V and Sivasubrahmanian, V. (2001).Studies on the kinetics of phosphate uptake by some freshwater micro algae. *Indian Hydrobiology Journal*.**4**(1)**:**1- 9.

Andrew,D.E.Lenore, S.C. Arnold, E.G. (1995).Standard Methods for the examination of water and waste water.19th Edition.Washington DC.

Andersen, L. L. (2012). Inner and outer life at work.The roots and horizon of psychoanalytically informed work life research. Forum Qualitative Sozialforschung / Forum*: Qualitative Social Research*. **13**(3)**:** 26-36

APHA**. (**2005) Standard Methods for the Examination of Water and Waste water.American Public Health Association Publication. 21st Edition, Washington DC**.**

Arbib, Z., Ruiz, J., Álvarez-Díaz, P., Garrido-Pérez, C. and Perales, J. A. (2014).Capability of different microalgae species for phytoremediation processes: Waste water tertiary treatment, CO_2 bio-fixation and low cost biofuels production. Water research. **49 (3):**465-474.

Ayodhya, D.K. (2013). Bioremediation of waste water by using microalgae: an experimental study. *International Journal of Life Sciences.Biotechnology & Pharmaceuticals Research.***2**(3)**:**2-10.

Ayyasamy, P. M. Yasodha, R..S. Rajakumar, S.Lakshmanaperumalsamy, P. Lee, S. and Rahman, P.K.(2008).Impact of sugar factory effluent on the growth and biochemical characteristics of terrestrial and aquatic plants.*Bulletin of Environmental Contamination and Toxicology.***81**(5)**:**449-454.

Azarpira, H. Behdarvand, P. Dhumal, K. and Pondhe, G. (2014).Potential use of Cyanobacteria species in phycoremediation of municipal water.*International Journal of biosciences.* **4**(4)**:**105-111.

Aziz, M.A and Nag, W.G(2003): Industrial waste water treatment using an activated algae-reactor;*Water Science Technology Journal.***28**(1)**:**71-76.

Becker, E.W. (1994). Microalgae: Biotechnology and microbiology. PP: 293. Cambridge University Press 1st Edition. United Kingdom.

Benemann, J.R. Van Olst, J.C. Massingill, M.J. Weissman, J.C.and Brune, D.E.(2002). The Controlled Eutrophication Process: Using Microalgae for CO_2 Utilization and Agricultural Fertilizer Recycling. Report to the Electric Power Research Institute and U.S. Dept. of Energy.pp 1-7.

Bitton, G. (1990).Waste watermicrobiology; 4th Edition. Wiley-Blackwell, New York.

Borowitzka,M.A(1998). Limitsto growth. In: Wong Y-S and Tam,NFY (Eds.) Waste water Treatment with Algae.Springer-Verlag, New *York Science Journal*. **4:** 203-226.

Chan, K.Y. Wong, K.H and Wong, P.K (1979).Nitrogen and phosphorus removal from sewage effluent with high salinity by *Chlorella salina*. *Environmental Pollution Journal. **18:** 139 – 146.*

Chinnasamy, S. Bhatnagar, A. Claxton,R. Das, K. (2010). Biomass and bioenergy production potential of microalgae consortium in open and closed bioreactors using untreated carpet industry effluent as growth medium. *Bioresource Technology Journal.***101:** 6751-6760.

Colak. O.Kaya. Z (1988).A study on the possibilities of biological waste water treatment using algae. *Doga Biyolji Serisi.**12*** (1)**:**18–29

Costa, J.C. Oliveira, J.V. and Alves, M.M. (2013). Biochemical methane potential of brewery wastes and co-digestion with glycerol. *2nd International conference, Universidade do Minho, Portugal.*

De la Nou, E. J. De Pauw, N.(1988).The potential of microalgal biotechnology.A review of production and uses of microalgae.*Biotechnology Advances Journal*. **6**, 725-770.

Demirel, B. Scherer, P. Yenigun, O. and Onay, T.T. (2010). Production of Methane``` and Hydrogen from Biomass through conventional and High rate Anaerobic Digestion Processes.**40:**116-146.

Deshmukh, K. K. (2014). Environmental impact of sugar mill effluent on the quality of ground water from Sangamner, Ahmednagar, Maharashtra, India. *Research Journal of recent sciences.***3:**385-392.

Dominic, V.J. Murali, D.S. and Nisha, M.C. (2009).Phycoremediation efficiency of three microalgae *Chlorella vulgaris, Synechocystis salina and Gleocapsa gelatinosa*. 16: *Academic ReviewJournal*. **16:** 138-146.

Eldoret Water andSanitation Company water quality weekly reports (2015). Eldoret Water and Sanitation Company, Eldoret.

EL-Sheekh, M.M.EL-Naggar,A.H. Osman,M.E.H.(2015) .Comparative Studies on the Green Algae Chlorella homosphaera and *Chlorella vulgaris* with respect to oil pollution in the River Nile; *Water.Air and Soil pollution journal.* **124**(1)**:**187-204.

Elumalaei, S. Saravanan, G.K. Ramganesh, S. Sakhtival, R. Prakasam, V. (2013)Phycoremediation of textile dye industrial effluent from tirupur district, Tamil Nadu, *India. International Journal of Science Innovation and Discoveries***. 3:**31–37.

Enden, J.C. and Calvert, K.C. (2002).Review of coffee waste water characteristics and approaches to treatment.*PPP project, GTZ, Vietnam***.**1-10.

Environmental Management and Coordination Act 2009 (Kenya).Government printer, Kenya.

Ganapathy, S.G. Baskaran, R. Mohan, P.M. (2011) Microbial diversity and bioremediation of distilleries effluent. *Journal of Research in Biology*.**3**:153–162.

Gonzalez, L. E.Canizares, R.O.Baena, S. (1997).”Effeciency of ammoniaand phosphorus removal from a colombian agroindustrial waste waterby the microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus*”.*Bioresource Technology*.**60:** 259-262, 1997.

Gray, N.F.(1989). Biology of Waste water Treatment. Oxford University Press, Oxford.

Gregorio Di, S. Giorgetti.L. Ruffini, C. Mariotti, L.(2014) Phytoremediation for improving the quality of effluents from a conventional tannery waste
water treatment plant. *International Journal of Environmental Science and Technolog.***20:**24-40.

Hammouda, O. Gaber, A and Abdel-Raouf, N. (1994) Microalgae and waste water treatment .*Ecotoxicology and Environmental Safety Journal*.**31:** 205-210.

Hodaifa, G. Martínez, M.A. Sánchez, S. (2008) Use of industrial waste water from olive-oil extraction for biomass production of *Scenedesmus obliquus*. *Bioresource Technology Journal.* **99:** 1111-1117.

Hongtao,W. Tao, W. Bingru, Z. Fengting,L. Brahima, T. Isaiah, B. Omosa,T. Chiramba,M. Mahesh, P.(2013)Water and Waste water Treatment in Africa –Current Practices and Challenges; *www.cleanjournal.com Clean – Soil, Air, Water*.**42** (8)**:** 1029–1035.

Horan, N.J.(1990) Biological Waste water Treatment Systems. Theory and operation. John Wiley and Sons Ltd, England.

Huppe, H.C and Turpin, D.H (1994) Integration of carbon and nitrogen metabolism in plant andalgal cells. *Annual Reviewof Plant Physiology and PlantMolecular Biology*.**45:** 577-607.

Jayangouder, I.A.Thanecker,K.P. Krishnamurthi and satyanarayana, S.(1983).Growth potential of algae in anaerobically treated slaughter house waste. *Indian Journal of Environmental health.* **25**(3)**:**209-213.

Jaysudha,S. and Sampathkumar, P. (2013)**.**Nutrient removal from tannery effluent by free and imobilized cells of marine microalgae *Chlorella Salina. International Journal of Environmental Biology.***4** (1)**:**21-26.

Jenkins, D., Richard, M. and Daigger, G. (2003). *Manual on the Causes and Control of Activated Sludge Bulking and Foaming.* 2nd edition. Lewis publishers, Boca

Khazenzi, J. A, Odipo, O. Wakhisi, J. Raburu, P.(2013).Risk among consumers of nitrate contaminated groundwater in Langas, Eldoret, Kenya**;** *Baraton Interdisciplinary Research Journal*.**3**(2)**:** 41-50.

Khemka and Saraf (2015). Phycoremediation of dairy waste water coupled with biomass production using *Leptolyngbyasp*. **4(**3)**:** 104 – 111.

Kothari, R. Kumar, V,.Tyagi, V.V. (2012). Assessment of waste treatment and energy recovery from dairy industrial waste by anaerobic digestion.IIOAB, Special issue on Environmental Management for Sustainable development**. 2:** 1–6.

Kotteswari, M. Murugesan, S.J. Kamaleswari and Veeralakshmi, M. (2007).Biomanagement of diary effluent by using cyanobacterium.*Indian hydrobiology Journal*.**10**(1):109-116.

Kshirsagar, A.D. (2013) Bioremediation of waste water by using microalgae: an experimental study. *International Journal of Life Sciences Biotechnology and Pharmaceutical Research.***2**:140–146.

Kshirsagar, A.D**.(**2014). Bioremediation of waste water by using microalgae: An experimental study. *International Journal of Life Sciences Biotechnology and Pharmaceutical Research.***2:**338-346.

Kumar, S. S. and Saramma, A. V.(2012). Nitrate and Phosphate uptake by immobilized cells of *Gloeocapsa gelatinosa. Journal of the Marine Biological Association of India.***54** (1)**:** 119 -122.

Kurt, H and Jessica, L.(1995).Removal of Nutrients from Sewage Water in East Africa. Research and Capacity Building for Sustainable Coastal Management**. 24** (7)**:**510-512.

Larsdotter, K. (2006). Microalgae for phosphorus removal from waste water in a Nordicclimate.A doctoral thesis from the School of Biotechnology, Royal Institute of Technology,Sweden.

Lesmanaa, S.O. Febrianaa, N.F. Soetaredjoa, E. Sunarsob, J and Ismadjia, S. (2009). Studies on potential applications of biomass for the separation of heavy metals from water and waste water.*Biochemical Engineering Journal.***44:**19–41.

Li, Y.Chen, YF. Chen, P. Min, M. Zhou, W.G. (2011) Characterization of a microalgae *Chlorella sp.* well adapted to highly concentrated municipal waste water in nutrient removal and biodiesel production. *Bioresource Technology Journal.* **102:** 5138-5144.

Lim,S. Chu,W. S. Phang,S (2010).Use of *Chlorella vulgaris* for bioremediation of textile waste water; *Journal of Bioresources Technology.***101:** 7314–7322.

Maghanga, J.K. Segor, F.K.. Etiégni, L. and Lusweti, J. (2009). Electrocoagulation method for colour removal in tea effluent: a case study of chemomi tea factory in rift valley, Kenya. Bull. *Chemistry Society Journal of Ethiopia.***23:**371-381.

Mamun, A. A, Amid, A. Karim,A.I, and Rashid,S.S (2012). Phytoremediation of Partially Treated Waste water by *Chlorella vulgaris; International Conference on Chemical Processes and Environmental issues July 15-16, 2012* Singapore.

Manoharan, C. and Subrahmanian, G (1992).Interaction between peppermill effluent and the cyanobacterium *Oscillatoria Pseudogerminata* var. *unigranulata. Pollution Research Journal.* **11:**73– 84.

Marecik, R. Biegańska, R. Paweł, C. Łukasz, Ł. Łukasz, C. (2013).Phytoremediation of Industrial Waste water Containing Nitrates, Nitroglycerin, and Nitroglycol; *Polish Journal of Environmental Studies. **22(**3**):**773-780.

Martinez,M.E Sanchez, S. Jimenez, J.M, El-Yousfi, F. Munoz, L. (2000).Nitrogen and phosphorous removal from urban waste water by the microalgae *Scenedesmus obliquus*. *Journal of Technology.***73:**263-272.

Mostafa, E. Phang, S. Chu, W.(2012).Use of an algal consortium of five algae in the treatment of landfill leachate using the high-rate algal pond system. Journal *of Applied Phycology*.**24:**953–963

Mouchet, P.(1986).Algal reactions to mineral and organic micropollutants, ecological consequences and possibilities for industrial scale application; a review.*Water Research Journal.***20:** 399-412.

Muhammad, M. N. and Nwaedozie, J.M. (2011).Application of Marine Biomass for the Removal of Metals from Industrial Waste water. Proceedings of the 1 st. International

Technology, Education and Environment Conference African society for Scientific Research. (ASSR).**1:**376-380.

Murali, D.S. and Nisha, M.C. (2009).Phycoremediation efficiency of three micro algae *Chlorella vulgaris, Synechocystis salina and Gleocapsa gelatinosa*. Academic review.**16** (2)**:** 138-146

Nagendra, M. N. and Nwaedozie, J.M. (2011).Effect of industrial effluents and wastes on physicochemical parameters of river Rapti.*Pelagia research library*.**1:** 1-10.

Nanda, S. Sarangi, P.K. Abraham, J. (2010).Cynobacterial remediation of industrial effluents II.Paper mill effluents. *New York Science Journal*.**3:**37-41.

Nunez, V.J. Voltolina, D. Nieves, M. Pina, P. Medina, A and Guerrero, M. (2001) Nitrogen budget in *Scenedesmus obliquus* cultures with artificial waste water. *Bioresources.Technology Journal*.**78:**161-164.

Oilgae Guide (2009). A report on Algae-based Waste water treatment. *Food and Agriculture Organization Journal*. Pp 2-44.

Olguin, E.J.(2003). Phycoremediation:Key issues for cost-effective nutrient removal process.*Biotechnology Advances Journal*. **22:**81-91.

Olguín, E.J. (2012). Dual purpose microalgae-bacteria-based systems that treat waste water and produce biodiesel and chemical products within a biorefinery. *Biotechnology Advances Journal*. **30:** 1031 1046.

Oliver, R.L and Ganf, G.G (2000).In Whitton, B.A and Potts, M (Eds.).Freshwater Blooms. The Ecology of Cyanobacteria: Their Diversity in Time and Space. *Kluwer academic , Dordrecht.The Netherlands*. pp. 149-194.

Omosa, I. B. Wang, H. Cheng, S. Li, F. (2012) Sustainable Tertiary Waste water Treatment Required for Water Resources Pollution Control in Africa, *Environmental Science. Technology Journal*. **46** (13)**:** 7065–7066.

Oso, W. Y and Onen, D.(2009). A General Guide to Writing Research and Report.A Hand Book for Beginning Researchers, Second Edition, Makerere University, Kampala.ISBN9969748-3-0.

Oswald, W.J. Gotaas, H.B. Golueke, C.G. Kellen, W.R. (1957) Algae in waste treatment. *Sewage and Industrial WastesJournal*.**29:** 437-455.

Otuu, F. Inya-Agha, C. Stella, I. Clement,C. Chukwu, C. Chinyere, C. and Ani, U. G.(2014).Physico-chemical profile and phytoremediation of Ebony Paint Industrial Effluent using n-Hexane de-fatted Moringaoleifera Seed Powder; *IOSR Journal of Environmental Science, Toxicology and Food Technology*.
***8*(** 3)**:** 59-64.

Pavasant, P. R., Apiratikul, V., Sungkhum, P., Suthiparinyanont, S. and Wattanachira, T. F.(2006).“Biosorption of $Cu^{2+,}$ $Cd^{2+,}$ $Pb^{2+,}$ and Zn^{2+}using dried marine green *macroalga Caulerpa lentillifera’*. *Bioresources Technology Journal*. **97:** 2321-2329.

Perez-Garcia, O. Escalante, F.M. de-Bashan, L.E.Bashan, Y. (2011) Heterotrophic cultures of microalgae: metabolism and potential products. *Water Research Journal.* **45**: 11-36.

Raburu, P.O. and Okeyo, J.B. (2000).Impact of agro-industrial activities on the quality of river Nyando and the Lake Victoria basin, Kenya.pp 1-8, Moi University,Kenya.

Rajasri,Y.and Goutham, R.V.N.H. (2013).Two stage treatment of dairy effluent using immobilzed *chlorella pyrenoidosa. Yadavalli and Heggers journal of health sciences.* **1:** 1-6.

Rao, P.H, Kumar, R.R. Raghavan, B.G. Subramanian, V.V, Sivasubramanian, V. (2011). Application of phycoremedation technology in the treatment of waste water from a leather-processing chemical manufacturing facility *Water South Africa Journal* (*Water SA).* **37:** 7-14.

Rawat, I. Kumar, R.R. Mutanda, T. Bux, F.(2011).Dual role of microalgae: phycoremediation of domestic waste water and biomass production for sustainable biofuels production. *Applied Energy Journal.* **88:**3411–3424.

Richmond, A. (2004). Handbook of Microalgal Culture: Biotechnology and Applied Phycology. Blackwel science Ltd. Oxford, UK.

Rybicki, S. (1997). Advanced Waste water Treatment: Phosphorus removal from waste water. *Report No. 1-. Royal Institute of Technology, Stockholm, Sweden.*

Sahu, O. (2014). Reduction of organic and inorganic pollutants from waste waters by algae. *International journal of natural sciences.**8**: 1-8.*

Saranraj, P and Stella, D.(2012).Bioremediation of sugar mill effluent by Immobilized bacterial consortium. *International journal of Research in Pure and Applied Microbiology.**2**:43-48.*

Sashenka, F. M. P.Sanchez, S and Carmen, C.(2008). Nitrate and phosphate removal by chitosan immobilized *Scenedesmus. Bioresource Technology*.**99:**1274-1279.

Sawayama, S. K.K. Rao,S.and Hall,D.O(2000). Nitrate and phosphate ions removal from water by *Phormidium laminosum* immobilized on hollow fibres in a photobioreactor. *Applied .Microbiology and .Biotechnology Journal*. **49:**463-468.

Sedlak, R. (1991).*Phosphorus and Nitrogen Removal from Municipal Waste water: Principles and Practice* (2^{nd} Edn.). Lewis Publisher, Ann Arbor, Michigan.

Sengar, R.M.S, Singh, K.K. Singh, S. (2011). Application of phycoremediation technologyin the treatment of sewage water to reduce pollution load. *Indian Journal of Scientific Research.* **2:** 33-39.

Sharma, G.K. and Shakeel, A. K. (2013).Bioremediation of Sewage Waste water Using Selective Algae for Manure Production. *International Journal of Environmental Engineering and Management.* **4:** 573-580.

Shi, J. Podola, B. Melkonian, M. (2007). Removal of nitrogen and phosphorus from waste water using microalgae immobilized on twin layers*: Journal of Applied Phycology.***19** (5)**:**417–423.

Sivasubramanian, V. Subramanian, V. V. Raghavan, B. G. and Ranjithkumar, R. (2009) Large scale phycoremediation of acidic effluent from an alginate industry. *Science Asia Journal*.**35:** 220 – 226.

Sivasubramanian, V. (2010) Gaining an edge with algal technology. Search - The IndustrialSource book. **13** (3) **:** 6 - 80.

Sivasubramanian, V. Subramanian, V.V.and M Muthukumaran, M. (2012) Phycoremediation of effluent from a soft drink manufacturing industry with a special emphasis on nutrient removal – a laboratory study.*Journal of Algal Biomass. **3:** 21– 29.*

Sood, A. Uniyal, P.L. Prasanna, R. Ahluwalia, A.S. (2012) Phytoremediation potential of aquatic macrophyte, *Azolla*. *Ambio Journal*.**41:**122–137.

Sreesai, S and Pakpain. (2007).Phosphate and Nutrient recycling by *Chlorella vulgaris* from the Bangkok city, Thailand. *Science Asia Journal*.**33:**293-299.

Sydney, E.B. da Silva, T.E.Tokarski, A. Novak, A.C. de Carvalho, J.C. Woiciecohwski, A.L (2011) Screening of microalgae with potential for biodiesel production and nutrient removal from treated domestic sewage. *AppliedEnergy Journal*. **88**:3291–3294.

Tam, N.F.Wong, Y.S.(1989).Waste water nutrient removal by *Chlorella pyrenoidosa* and *Scenedesmus sp. Environmental Pollution Journal*.**58:** 19-34.

Tam, N.F. Wong, Y.S (1990).The comparison of growth and nutrient removal efficiency of *Chlorella pyrenoidosa* in settled and activated sewage. *Environmental Pollution Journal*.**65:** 93-108.

Tam, N.F.Y and Wong, Y.S.(2000).Effect of immobilized microalgal bead concentrations on waste water nutrient removal. *Environmental Pollution*. **107** (1)**:** 145-151.

Tebbutt, T.H.Y.(1983). Principles of water quality control.Pergammon Press, Oxford.

Van Larsdrecht, M.C. (2005). Role of biological processes in phosphate recovery. *Natural History Museum, London*.

Veele, W. (2012).The use of microalgae as method for phosphorus removal from a human derived waste stream, from a lab scale to a household scale cultivation system. University of Groningen. pp 2-66.

Vinod, K. and Chopra, A.K. (2010). Influence of sugar mill effluent on physicochemical characteristics of soil at Haridwar (Uttarakhand), India. *Journal of Applied and Natural science*.**2:**269– 279.

UNEP (2010).Africa Water Atlas.UNEP, Nairobi.

Wang, H. T. Li, F. T. Keller, A. A. Xu, R.(2009). Chemically Enhanced Primary Treatment (CEPT) for Removal of Carbon and Nutrients from Municipal Waste water Treatment Plants: A Case Study of Shanghai, *Water Science and Technology Journal***. 60** (7)**:**1803–1809.

Wang, L. Min, M. Li, Y.Chen, P. Chen, Y.(2010). Cultivation of green algae *Chlorella sp*.in different waste waters from municipal waste water treatment plant. *Applied Biochemistry and Biotechnology Journal.* **162:** 1174-1186.

Wang, Z. Ma, X. Zhou, W. Min, M. Cheng, Y. (2013). Oil crop biomass residue-based media for enhanced algal lipid production. *Applied Biochemistry and Biotechnology Journal.***171:** 689-703.

Weerawattanaphong,W.(1998); Nutrients reductions from poutry waste water by green algae; *Chlorella vulgaris*; MSc thesis in Environmental technology; Bangkok Faculty of Graduate studies, Mahidol University, Thailand.

Wells, C.D. Dekker, L.G.Clark, S. Hart, O.O.Neba, A. Rose,P.D. (2013). Integrated algal ponding systems (IAPS) in the treatment of domestic waste water: a nine year performance evaluation; Environmental Biotechnology Research Unit (EBRU). Rhodes University, Grahamstown,South Africa.

World Health Organization (1970).European Standards for Drinking Water,2nd edition. World Health Organization, Geneva.

WHO (2006). *Guidelines for the Safe Use of Waste water, Excreta and Greater.* Vol. 3. World Health Organisation Press, Geneva, Switzerland.

Yamane, T (1991). Statistics, An introductory Analysis 2nd edition. Harper and Row, New York.

Yoshida, N.I.K., Okuno, T. and Tanaka, K.(2006) "Purification and Characterization of Cadmium-Binding Protein from Unicelluar Algae Chlorella sorokinian". *Current Microbiology Journal.* **52(**6)**:**460–463.

Yu, I.T. Su, J.J. Wu, J.F.Lee, S.L. Ju, C.C, Yen, H.T.(2005) Dietary modification for reducing electrical conductivity of piggery. *Waste water Journal*.**18(**9)**:**1343-1347.

Zhao, B., Zhang, Y., Xiong, K., Zhang, Z., Hao, X. and Liu, T.(2016). Effect of cultivation mode on microalgal growth and CO2 fixation. Chemical Engineering Research and Design.**89**(9)**:** 1758-1762

Zhou,W. Cheng, Y. Li, Y. Wan, Y. Liu, Y. (2012c) Novel fungal pelletization-assisted technology for algae harvesting and waste water treatment. *Applied Biochemistry and Biotechnology*.167**:** 214-228.

Zhou, W. Li, Y. Min, M. Hu, B. Chen, P. (2011) Local bioprospecting for high-lipid producing microalgal strains to be grown on concentrated municipal waste water for biofuel production.. *Bioresources Technology Journal.* **102**: 6909-6919.

Zhou,W.G. Li, Y. Min, M. Hu, B. Zhang, H.(2012a) Growing waste water-born micro-algal *auxeno chlorella protothecoides* UMN280 on concentrated municipal waste water for simultaneous nutrient removal and energy feedstock production. *Applied Energy Journal*.**98:** 433-440.

Zhou, WG. Min, M. Li, Y.Hu, B. Ma, X. (2012b) A hetero-photoautotrophic two-stage cultivation process to improve waste water nutrient removal and enhance algal lipid accumulation, *Bioresource Technology Journal*. **110:** 448-455.

APPENDICES

APPENDIX I:APPARATUS, EQUIPMENT AND REAGENTS USED IN pH ESTIMATION.

a) pH meter: Consisted of potentiometer, a glass electrode, a reference electrode and atemperature compensating device. A balanced circuit was completed through potentiometer when the electrodes were immersed in the test solution.

b) Reference electrode: Consisted of a half cell that provided a standard electrode potential.Generally calomel, silver-silver chloride electrodes were used as reference electrode.

c) Sensor (glass) electrode: The glass electrode consisted essentially of a very thick walled glass bulb, made of low melting point glass of highelectrical conductivity, blown at the end of a glass tube. This bulb contained an electrode, which had a constant potential, e.g. a platinum wire inserted in a solution of H+ hydrochloricacid saturated with quinhydrone. The bulb was placed in the liquid where pH was determined.

d) Beakers:

e) Magnetic Stirrer:

Reagents and standards

a) pH 4 buffer solution was prepared by dissolving 10.12g of potassium hydrogen phthalate, $KHC_8H_4O_9$ in distilled water. Dilute to 1L.

b) pH 7 buffer solution was prepared by dissolving 1.361g of anhydrous potassium dihydrogen phosphate, KH_2PO_4, and 1.42g anhydrous disodium hydrogen phosphate, Na_2HPO_4, which had been dried at 110°C and the mixture diluted to one litre with boiled and cooled distilled water.

c) pH 9.2 buffer solution was prepared by dissolving 3.81gmof, $Na_2B_4O_7.10H_2O$ in distilled water, which has been previously boiled and cooled then the mixture dilutedto 1L.

APPENDIX II: APPARATUS, EQUIPMENT AND PROCEDURE FOR CONDUCTIVITY

The apparatus and equipment included a Conductivity meter and Conductivity Cells: The cell choice depended on the expected range of conductivity and the resistance range of the instrument. Experimentally the range of the instruments assembly was checked by comparing the instrumental results with the true conductance of the potassium chloride solution (APHA 2005).

Procedure for taking conductivity measurements

1. The probe was rinsed with distilled water before use to remove any impurities adhering to the probe body and shaken to dryness and to avoid contamination or dilution of the waste water sample the probe was rinsed with a small volume of the sample liquid.
2. The conductivity meter was turned on by pressing the ON button.
3. The meter probe was dipped into the waste water sample.
4. Time was allowed for the waste water sample to stabilize and the reading on the display recorded.When dipping the meter probe into the waste water sample care was taken to ensure that the liquid level was above the upper steel band. Then gently the waste water sample was stirred with the meter probe inorder to create a homogenous waste water sample.

   When the value of the solution being measured was higher than the range selected (Or) appeared on the primary display, then RANGE was pressed until the correct range was selected.The meter resets to the Auto-ranging function once it is turned off. The manual ranging function was reset each time the meter was turned on (APHA 2005).

APPENDIX III: CHEMICAL OXYGEN DEMAND BY REFLUX METHOD APPARATUS AND REAGENTS;

Apparatus

- Electrothermal kit
- Reflux apparatus

Reagents

- Standard potassium dichromate solution (0.25N)

  Dissolve 12.25g of $K_2Cr_2O_7$ (dried at 103 degrees centigrade for 2 hrs) and dilute to 1 litre.
- Concentrated H_2SO_4 with 22g Ag_2SO_4 added
- Standard ferrous ammonium sulphate (0.1N)

  Dissolve 39.213g $Fe(NH_4)_2SO_4.6H_2O$ in distilled water. Add 20ml conc H_2SO_4 cool and dilute to 1 litre.
- Ferroin indicator

  Prepared by Dissolving 1.735 1,10 phenanthroline dehydrate with 695mg ferrous sulphate ($FeSO_4.7H_2O$) in 1 litre of distilled water.

APPENDIX IV: APPARATUS AND EQUIPMENT USED IN TOTAL DISSOLVED SOLIDS DETERMINATION

Evaporatory dish (porcelain) 100/200mL.Drying oven – equipped with thermostatic control capable of maintaining the temperature within 2°C range. Desiccator – provided with desiccants Analytical balance – 200mg capacity of weighing to 0.1mg.Filter holder – Gooch crucible adapter or membrane filters.Suction flask – 500mL capacity

APPENDIX V: NITRATE ESTIMATION APPARATUS AND EQUIPMENT

a. Spectrophotometer,

b. A Filter.

i) Membrane filter: 0.45μm membrane filter,

ii) Paper: Acid-washed, ashless hard-finish filter paper which can hold most of the fine precipitates.

c. Nessler tubes, 50mL (APHA 2005)

APPENDIX VI: RESEARCH PERMIT NANDI COUNTY

REPUBLIC OF KENYA

MINISTRY OF HEALTH

Telephone 053 52233
When replying
Please quote

County Director of Health
Nandi County
P.O. Box 5
KAPSABET.

9/11/2015

Our Ref
To
SCPHO-Chesumei
Emgwen
Nandi Hills
Aldai

RE: ALEXANDER M MBEKE:PHO STUDENT FROM MASENO UNIVERSITY

The above officer reported on 9/11/2015 for research work which has to be carried out in the tea factories within the following sub- counties, Aldai, Emgwen, Chesumei and Nandi Hills.

With this regard i am informing all the sub-county Public Health Officers in the mentioned areas to accord him the necessary support and introduce him to the factories within your area of jurisdiction.

Thank you.

COUNTY PUBLIC HEALTH OFFICER
NANDI COUNTY
09 NOV 20
P. O. Box 802-30300,
KAPSABET

MR ALFRED BICHIY
COUNTY PUBLIC HEALTH OFFICER
NANDI COUNTY

APPENDIX VII: RESEARCH PERMIT KAKAMEGA COUNTY

REPUBLIC OF KENYA

COUNTY GOVERNMENT OF KAKAMEGA
OFFICE OF THE HEAD,PUBLIC HEALTH & SANITATION

Telephone: 056 31125
Fax: 056 31125
E-mail:pdmswesternh@gmail.com
When replying please quote

KAKAMEGA COUNTY
P. O. BOX 359 - 50100
KAKAMEGA

Our Ref:

Date: 12TH October, 2015

To;
SUB COUNTY PUBLIC HEALTH OFFICERS:

- ***Mumias East***
- ***Malava***

RE: ALEXANDER M. MBEKE: PhD STUDENT FROM MASENO UNIVERSITY.

TAKE NOTE that the above mentioned officer shall be undertaking his research work **"Phycoremediation efficacy of *Chlorella vulgaris, Synechocystissalina* and *Gloecapsagelatinosa*using waste water from coffee, tea and sugar factories in Kenya"**In Kakamega County.

Your area has been chosen because of favorable conditions to undertake the study and the benefits that shall be accrued from the study. This office has given him the express authority to carry out the same. Therefore accord him all the necessary support and introduce him to the Factory.

Paul N. Manyasi
Head of Public Health and Sanitation
Kakamega County

APPENDIX VIII: RESEARCH PERMIT BUNGOMA COUNTY

REPUBLIC OF KENYA

THE COUNTY PUBLIC HEALTH OFFICE

P0 BOX 383,

BUNGOMA.

12/10/2015

TO

THE SUB COUNTY PUBLIC HEALTH OFFICER

CHEPTAIS SUB COUNTY

Dear; Madam

RE: MR ALEXANDER .M.MBEKE -PHD STUDENT MASENO UNIVERSITY

This is to confirm that the student in regard chose to undertake research in your sub county to meet course objective. The purpose of this letter is to inform you to accord him necessary support to undertake his research. Any issue of concern about the student can be communicated to this office for clarity.

Thank you

Robert Wetoto

DEPUTY COUNTY PUBLIC HEALTH OFFICER

BUNGOMA.

APPENDIX IX: RESEARCH PERMIT ELDORET WATER AND SANITATION COMPANY

3rd February, 201

Date _____

Your Ref _____

Our Ref ELDOWAS/ADM/23/1A/VOL. XIX/120

The Dean
School of Public Health & Community Development
Private Bag
MASENO.

Dear Sir

RE: RESEARCH STUDY –ALEXANDER MBEKE –PG/PHD/0003/2013

Reference is made to your letter of 30th October 2014 concerning the above subject.

This is to confirm that permission is hereby granted to **Mr. Alexander Mbeke PG/PHD/0003/2013,** a student in your institution to carry out a research study entitled: **Phycoremediation efficacy of Chlorella vulgaris, Synecholcystis salina and Gloecapsa gelatinosa,** in Eldowas.

Please note that the information gathered from this study should only be used for learning purposes.

Yours faithfully
ELDORET WATER & SANITATION CO. LTD

PERIS TENAI
FOR: HUMAN RESOURCE & ADMIN.MANAGER

cc
Mr. Alexander Mbeke
PG/PHD/0003/2013

Eldoret Water and Sanitation Company Limited
P.O.Box 8418; Phone (053) 2063403 / 2061915; Fax (053) 2063556; Email info@eldowas.org
Mission: Eldowas is committed to providing quality and adequate water services in a cost effective manner to its stakeholders by qualified and motivated human resource.

APPENDIX X: SIGNIFICANCE OF PHYCOREMEDIATION EFFICACY BETWEEN DAYS (T-TESTS)

DAY0 AND DAY5

Variable	SPECIES=*S.salina*, EFFLUENT TYPE=Coffee T-tests; Grouping: DAY (Original Data) Group 1: Day0 Group 2: Day5										
	Mean	Mean	t-value	Df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	734.875	306.250	1.68688	14	0.113772	8	8	663.324	276.606	5.750802	0.034373
PH	6.500	7.313	-1.71914	14	0.107611	8	8	1.069	0.803	1.774328	0.467041
NITRATE (mg/L)	21.375	8.575	5.20479	14	0.000133	8	8	6.022	3.481	2.993279	0.171307
PHOSPHATE (mg/L)	4.419	2.511	0.85387	14	0.407553	8	8	5.045	3.804	1.758789	0.473816
BOD (mg/L)		1147.000		6		0	8		2376.847	0.000000	1.000000
COD (mg/L)	3459.000	2331.125	0.51777	14	0.612705	8	8	5304.451	3134.360	2.864066	0.188438
CONDUCTIVITY (µs/cm)	1185.250	493.625	1.68791	14	0.113569	8	8	1069.768	445.830	5.757594	0.034263

Variable	SPECIES=*C. vulgaris*, EFFLUENT TYPE=Coffee T-tests; Grouping: DAY (Original Data) Group 1: Day0 Group 2: Day5										
	Mean	Mean	t-value	Df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	734.875	183.875	2.27927	14	0.038846	8	8	663.324	165.901	15.98658	0.001641
PH	6.500	7.163	-1.44332	14	0.170933	8	8	1.069	0.737	2.10596	0.346946
NITRATE (mg/L)	21.375	7.675	5.61974	14	0.000063	8	8	6.022	3.358	3.21625	0.146108
PHOSPHATE (mg/L)	4.419	2.050	1.11534	14	0.283486	8	8	5.045	3.261	2.39412	0.272131
BOD (mg/L)		1147.000		6		0	8		2376.847	0.00000	1.000000

Variable	SPECIES=*C. vulgaris*, EFFLUENT TYPE=Coffee T-tests; Grouping: DAY (Original Data) Group 1: Day0 Group 2: Day5										
	Mean	Mean	t-value	Df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
COD (mg/L)	3459.000	2285.750	0.54198	14	0.596351	8	8	5304.451	3057.998	3.00889	0.169372
CONDUCTIVITY (µs/cm)	1185.250	296.625	2.27938	14	0.038838	8	8	1069.768	267.368	16.00892	0.001633

Variable	SPECIES= *G.gelatinosa* EFFLUENT TYPE=Coffee T-tests; Grouping: DAY (Original Data) Group 1: Day0 Group 2: Day5										
	Mean	Mean	t-value	Df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	734.875	367.625	1.40076	14	0.183060	8	8	663.324	331.519	4.003459	0.087438
PH	6.500	7.650	-2.39050	14	0.031435	8	8	1.069	0.842	1.612903	0.543514
NITRATE (mg/L)	21.375	11.012	4.09110	14	0.001101	8	8	6.022	3.881	2.408481	0.268953
PHOSPHATE (mg/L)	4.419	2.932	0.64455	14	0.529641	8	8	5.045	4.138	1.486745	0.613763
BOD (mg/L)		1147.000		6		0	8		2376.847	0.000000	1.000000
COD (mg/L)	3459.000	2345.500	0.50791	14	0.619424	8	8	5304.451	3211.344	2.728396	0.208809
CONDUCTIVITY (µs/cm)	1185.250	592.875	1.40094	14	0.183008	8	8	1069.768	534.751	4.001994	0.087516

Variable	SPECIES=.*S. salina*, EFFLUENT TYPE=Tea T-tests; Grouping: DAY (Original Data) Group 1: Day0 Group 2: Day5										
	Mean	Mean	t-value	Df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P

Variable	SPECIES=*S. salina*, EFFLUENT TYPE=Tea T-tests; Grouping: DAY (Original Data) Group 1: Day0 Group 2: Day5										
	Mean	Mean	t-value	Df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	505.875	169.194	5.25342	30	0.000011	16	16	235.949	100.223	5.542391	0.001986
PH	7.711	9.017	-4.21708	30	0.000210	16	16	1.176	0.387	9.253006	0.000097
NITRATE (mg/L)	4.119	2.274	3.01889	30	0.005140	16	16	2.185	1.094	3.988795	0.011040
PHOSPHATE (mg/L)	69.196	31.600	5.61686	30	0.000004	16	16	23.155	13.441	2.967732	0.042908
BOD (mg/L)		684.500		14		0	16		300.952	0.000000	1.000000
COD (mg/L)	1777.813	1119.937	1.80786	30	0.080664	16	16	1057.632	1000.071	1.118427	0.831245
CONDUCTIVITY (µs/cm)	815.625	272.875	5.25205	30	0.000011	16	16	380.391	161.774	5.528936	0.002012

Variable	SPECIES=*C. vulgaris*, EFFLUENT TYPE=Tea T-tests; Grouping: DAY (Original Data) Group 1: Day0 Group 2: Day5										
	Mean	Mean	t-value	Df	P	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	505.875	105.019	6.64859	30	0.000000	16	16	235.949	49.9011	22.35703	0.000000
PH	7.711	9.024	-4.24899	30	0.000192	16	16	1.176	0.3776	9.70713	0.000072
NITRATE (mg/L)	4.106	1.798	3.82534	30	0.000616	16	16	2.180	1.0349	4.43846	0.006465
PHOSPHATE (mg/L)	69.196	29.853	5.95837	30	0.000002	16	16	23.155	12.7050	3.32153	0.026167
BOD (mg/L)		652.625		14		0	16		298.8054	0.00000	1.000000
COD (mg/L)	1777.813	1176.563	1.72988	30	0.093929	16	16	1057.632	902.3667	1.37374	0.546173

Variable	SPECIES=*C. vulgaris*, EFFLUENT TYPE=Tea T-tests; Grouping: DAY (Original Data) Group 1: Day0 Group 2: Day5										
	Mean	Mean	t-value	Df	P	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
CONDUCTIVITY (µs/cm)	815.625	188.187	6.41183	30	0.000000	16	16	380.391	92.2823	16.99117	0.000002

Variable	SPECIES=*G. gelatinosa*, EFFLUENT TYPE=Tea T-tests; Grouping: DAY (Original Data) Group 1: Day0 Group 2: Day5										
	Mean	Mean	t-value	Df	P	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	505.875	192.519	4.64219	30	0.000064	16	16	235.949	131.272	3.230666	0.029634
PH	7.711	9.040	-4.25013	30	0.000191	16	16	1.176	0.424	7.698522	0.000299
NITRATE (mg/L)	4.106	2.794	1.98967	30	0.055811	16	16	2.180	1.484	2.159137	0.147421
PHOSPHATE (mg/L)	69.071	34.922	5.05008	30	0.000020	16	16	23.069	14.120	2.669209	0.066538
BOD (mg/L)		652.625		14		0	16		298.805	0.000000	1.000000
COD (mg/L)	1777.813	1362.375	1.14118	30	0.262823	16	16	1057.632	1000.920	1.116532	0.833764
CONDUCTIVITY (µs/cm)	815.625	332.875	4.47567	30	0.000102	16	16	380.391	203.584	3.491182	0.020837

Variable	SPECIES=*S. salina*, EFFLUENT TYPE=Sugar T-tests; Grouping: DAY (Original Data) Group 1: Day0 Group 2: Day5										
	Mean	Mean	t-value	Df	P	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	474.500	285.500	10.69145	2	0.008635	2	2	17.678	17.6777	1.000000	1.000000

Variable	SPECIES=*S. salina*, EFFLUENT TYPE=Sugar T-tests; Grouping: DAY (Original Data) Group 1: Day0 Group 2: Day5										
	Mean	Mean	t-value	Df	P	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
PH	6.550	8.250	-3.30237	2	0.080745	2	2	0.636	0.3536	3.240000	0.645658
NITRATE (mg/L)	28.000	10.000	4.99230	2	0.037860	2	2	2.828	4.2426	2.250000	0.748668
PHOSPHATE (mg/L)	5.000	2.350	2.74424	2	0.111094	2	2	1.273	0.4950	6.612245	0.472233
BOD (mg/L)		880.000		0		0	2		28.2843	0.000000	1.000000
COD (mg/L)	4872.000	2777.500	1.72848	2	0.226044	2	2	1538.664	754.4829	4.159000	0.580466
CONDUCTIVITY (µs/cm)	765.000	460.500	10.63198	2	0.008731	2	2	28.284	28.9914	1.050625	0.984282

Variable	SPECIES=*C. vulgaris*, EFFLUENT TYPE=Sugar T-tests; Grouping: DAY (Original Data) Group 1: Day0 Group 2: Day5										
	Mean	Mean	t-value	Df	P	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	474.500	198.500	19.58975	2	0.002596	2	2	17.678	9.19239	3.6982	0.610543
PH	6.550	8.500	-4.23014	2	0.051597	2	2	0.636	0.14142	20.2500	0.278418
NITRATE (mg/L)	28.000	9.250	4.91341	2	0.039014	2	2	2.828	4.59619	2.6406	0.702389
PHOSPHATE (mg/L)	5.000	2.050	2.93173	2	0.099315	2	2	1.273	0.63640	4.0000	0.590334
BOD (mg/L)		880.000		0		0	2		28.28427	0.0000	1.000000
COD (mg/L)	4872.000	1289.000	3.29150	2	0.081216	2	2	1538.664	49.49747	966.3216	0.040945
CONDUCTIVITY (µs/cm)	765.000	320.000	19.90100	2	0.002515	2	2	28.284	14.14214	4.0000	0.590334

Variable	SPECIES=*G. gelatinosa*, EFFLUENT TYPE=Sugar T-tests; Grouping: DAY (Original Data) Group 1: Day0 Group 2: Day5										
	Mean	Mean	t-value	Df	P	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	474.500	320.500	12.23224	2	0.006617	2	2	17.678	2.121	69.4444	0.152062
PH	6.550	8.450	-4.00555	2	0.057046	2	2	0.636	0.212	9.0000	0.409666
NITRATE (mg/L)	28.000	11.500	5.15373	2	0.035648	2	2	2.828	3.536	1.5625	0.859107
PHOSPHATE (mg/L)	5.000	2.800	2.38624	2	0.139732	2	2	1.273	0.283	20.2500	0.278418
BOD (mg/L)		880.000		0		0	2		28.284	0.0000	1.000000
COD (mg/L)	4872.000	3590.500	0.95747	2	0.439370	2	2	1538.664	1102.379	1.9482	0.791551
CONDUCTIVITY (µs/cm)	765.000	518.000	12.28871	2	0.006557	2	2	28.284	2.828	100.0000	0.126902

DAY5 AND DAY10

Variable	SPECIES= *S. salina*, EFFLUENT TYPE=Coffee T-tests; Grouping: DAY (Original Data) Group 1: Day5 Group 2: Day10										
	Mean	Mean	t-value	Df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	306.250	243.125	0.488151	14	0.633000	8	8	276.606	239.306	1.33603	0.711964
PH	7.313	7.300	0.031410	14	0.975386	8	8	0.803	0.789	1.03412	0.965840
NITRATE (mg/L)	8.575	5.838	1.981399	14	0.067536	8	8	3.481	1.776	3.84148	0.096635
PHOSPHATE (mg/L)	2.511	2.040	0.271510	14	0.789961	8	8	3.804	3.103	1.50304	0.604118
BOD (mg/L)	1147.000	212.625	1.101950	14	0.289067	8	8	2376.847	320.095	55.13716	0.000027
COD (mg/L)	2331.125	2223.875	0.068484	14	0.946368	8	8	3134.360	3129.831	1.00290	0.997054
CONDUCTIVITY (µs/cm)	493.625	392.250	0.486250	14	0.634313	8	8	445.830	385.951	1.33436	0.713147

Variable	SPECIES=*C. vulgaris*, EFFLUENT TYPE=Coffee T-tests; Grouping: DAY (Original Data) Group 1: Day5 Group 2: Day10										
	Mean	Mean	t-value	df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	183.875	158.875	0.322053	14	0.752171	8	8	165.901	143.822	1.33060	0.715826
PH	7.163	7.175	-0.033507	14	0.973744	8	8	0.737	0.755	1.05166	0.948730
NITRATE (mg/L)	7.675	5.688	1.387503	14	0.186979	8	8	3.358	2.267	2.19454	0.321502
PHOSPHATE (mg/L)	2.050	1.641	0.279785	14	0.783734	8	8	3.261	2.538	1.64995	0.524718
BOD (mg/L)	1147.000	212.000	1.102649	14	0.288774	8	8	2376.847	320.715	54.92411	0.000028
COD (mg/L)	2285.750	2165.250	0.079143	14	0.938039	8	8	3057.998	3032.165	1.01711	0.982719
CONDUCTIVITY (µs/cm)	296.625	238.125	0.459138	14	0.653183	8	8	267.368	241.633	1.22435	0.796262

Variable	SPECIES=*G. gelatinosa*, EFFLUENT TYPE=Coffee T-tests; Grouping: DAY (Original Data) Group 1: Day5 Group 2: Day10										
	Mean	Mean	t-value	Df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	367.625	306.875	0.375962	14	0.712580	8	8	331.519	314.601	1.11044	0.893636
PH	7.650	7.650	0.000000	14	1.000000	8	8	0.842	0.842	1.00000	1.000000
NITRATE (mg/L)	11.012	8.088	1.746700	14	0.102581	8	8	3.881	2.716	2.04167	0.366973
PHOSPHATE (mg/L)	2.932	2.383	0.299574	14	0.768904	8	8	4.138	3.120	1.75851	0.473940
BOD (mg/L)	1147.000	213.000	1.101522	14	0.289247	8	8	2376.847	319.860	55.21815	0.000027
COD (mg/L)	2345.500	2267.250	0.048790	14	0.961776	8	8	3211.344	3203.949	1.00462	0.995304
CONDUCTIVITY (µs/cm)	592.875	487.375	0.402780	14	0.693193	8	8	534.751	512.735	1.08772	0.914531

Variable	SPECIES=*S. salina*, EFFLUENT TYPE=Tea T-tests; Grouping: DAY (Original Data) Group 1: Day5 Group 2: Day10										
	Mean	Mean	t-value	df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	169.194	131.938	1.106197	30	0.277433	16	16	100.223	90.0240	1.239425	0.682970
PH	9.017	8.698	2.315202	30	0.027626	16	16	0.387	0.3920	1.027499	0.958797
NITRATE (mg/L)	2.274	1.809	1.323346	30	0.195718	16	16	1.094	0.8853	1.527427	0.421521
PHOSPHATE (mg/L)	31.600	30.208	0.298418	30	0.767441	16	16	13.441	12.9509	1.077131	0.887483
BOD (mg/L)	684.500	482.437	1.812485	30	0.079931	16	16	300.952	329.0674	1.195567	0.733885
COD (mg/L)	1119.937	1124.625	-0.014103	30	0.988842	16	16	1000.071	876.1023	1.303023	0.614699
CONDUCTIVITY (µs/cm)	272.875	212.813	1.106106	30	0.277472	16	16	161.774	144.9359	1.245853	0.675774

Variable	SPECIES=*C. vulgaris*, EFFLUENT TYPE=Tea T-tests; Grouping: DAY (Original Data) Group 1: Day5 Group 2: Day10										
	Mean	Mean	t-value	df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	105.019	81.813	1.566550	30	0.127709	16	16	49.9011	31.9525	2.4390	0.094610
PH	9.024	8.642	2.921665	30	0.006557	16	16	0.3776	0.3604	1.0980	0.858720
NITRATE (mg/L)	1.798	4.819	-0.916552	30	0.366687	16	16	1.0349	13.1446	161.3141	0.000000
PHOSPHATE (mg/L)	29.853	28.870	0.222002	30	0.825817	16	16	12.7050	12.3435	1.0594	0.912448
BOD (mg/L)	652.625	443.750	1.906845	30	0.066158	16	16	298.8054	320.4654	1.1502	0.789889
COD (mg/L)	1176.563	1005.063	0.550826	30	0.585832	16	16	902.3667	858.3473	1.1052	0.848950
CONDUCTIVITY (µs/cm)	188.187	131.750	2.133128	30	0.041211	16	16	92.2823	51.8080	3.1728	0.032108

Variable	SPECIES=*G. gelatinosa*, EFFLUENT TYPE=Tea T-tests; Grouping: DAY (Original Data) Group 1: Day5 Group 2: Day10										
	Mean	Mean	t-value	Df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	192.519	174.000	0.424360	30	0.674331	16	16	131.272	115.0559	1.301741	0.616011
PH	9.040	8.731	2.038391	30	0.050410	16	16	0.424	0.4328	1.041744	0.937920
NITRATE (mg/L)	2.794	2.426	0.751478	30	0.458220	16	16	1.484	1.2797	1.344401	0.573686
PHOSPHATE (mg/L)	34.922	32.754	0.446477	30	0.658459	16	16	14.120	13.3388	1.120606	0.828358
BOD (mg/L)	652.625	502.125	1.391840	30	0.174205	16	16	298.805	312.7137	1.095259	0.862444
COD (mg/L)	1362.375	1219.375	0.409035	30	0.685420	16	16	1000.920	976.5864	1.050454	0.925316
CONDUCTIVITY (µs/cm)	332.875	280.500	0.760968	30	0.452616	16	16	203.584	185.3307	1.206683	0.720678

Variable	SPECIES=*S. salina*, EFFLUENT TYPE=Sugar T-tests; Grouping: DAY (Original Data) Group 1: Day5 Group 2: Day10										
	Mean	Mean	t-value	Df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	285.500	177.000	7.310916	2	0.018200	2	2	17.6777	11.3137	2.44141	0.724872
PH	8.250	8.150	0.342997	2	0.764298	2	2	0.3536	0.2121	2.77778	0.688084
NITRATE (mg/L)	10.000	4.500	1.739253	2	0.224120	2	2	4.2426	1.4142	9.00000	0.409666
PHOSPHATE (mg/L)	2.350	1.550	1.403293	2	0.295639	2	2	0.4950	0.6364	1.65306	0.841666
BOD (mg/L)	880.000	420.000	5.578319	2	0.030666	2	2	28.2843	113.1371	16.00000	0.311917
COD (mg/L)	2777.500	1250.000	2.611122	2	0.120688	2	2	754.4829	339.4113	4.94136	0.538245
CONDUCTIVITY (µs/cm)	460.500	285.500	7.288504	2	0.018309	2	2	28.9914	17.6777	2.68960	0.697178

Variable	SPECIES=*C. vulgaris*, EFFLUENT TYPE=Sugar T-tests; Grouping: DAY (Original Data) Group 1: Day5 Group 2: Day10										
	Mean	Mean	t-value	Df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	198.500	121.5000	10.43020	2	0.009067	2	2	9.19239	4.94975	3.44898	0.628906
PH	8.500	8.3500	1.34164	2	0.311753	2	2	0.14142	0.07071	4.00000	0.590334
NITRATE (mg/L)	9.250	4.5000	1.44454	2	0.285431	2	2	4.59619	0.70711	42.25000	0.194359
PHOSPHATE (mg/L)	2.050	1.3500	0.98504	2	0.428452	2	2	0.63640	0.77782	1.49383	0.873098
BOD (mg/L)	880.000	195.0000	33.22738	2	0.000905	2	2	28.28427	7.07107	16.00000	0.311917
COD (mg/L)	1289.000	613.0000	11.22620	2	0.007842	2	2	49.49747	69.29646	1.96000	0.789726
CONDUCTIVITY (µs/cm)	320.000	196.0000	11.09090	2	0.008032	2	2	14.14214	7.07107	4.00000	0.590334

Variable	SPECIES=*G. gelatinosa*, EFFLUENT TYPE=Sugar T-tests; Grouping: DAY (Original Data) Group 1: Day5 Group 2: Day10										
	Mean	Mean	t-value	Df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	320.500	196.000	69.06017	2	0.000210	2	2	2.121	1.4142	2.25000	0.748668
PH	8.450	8.400	0.14907	2	0.895172	2	2	0.212	0.4243	4.00000	0.590334
NITRATE (mg/L)	11.500	6.500	1.96116	2	0.188893	2	2	3.536	0.7071	25.00000	0.251332
PHOSPHATE (mg/L)	2.800	2.350	1.11631	2	0.380414	2	2	0.283	0.4950	3.06250	0.660997
BOD (mg/L)	880.000	543.000	2.83916	2	0.104897	2	2	28.284	165.4630	34.22250	0.215564
COD (mg/L)	3590.500	1724.500	2.06221	2	0.175295	2	2	1102.379	649.8311	2.87780	0.678189
CONDUCTIVITY (µs/cm)	518.000	315.500	81.00000	2	0.000152	2	2	2.828	2.1213	1.77778	0.819331

DAY10 AND DAY15

Variable	SPECIES=*S. salina*, EFFLUENT TYPE=Coffee T-tests; Grouping: DAY (Original Data) Group 1: Day10 Group 2: Day15										
	Mean	Mean	t-value	Df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	243.125	243.125	0.000000	14	1.000000	8	8	239.306	239.306	1.000000	1.000000
PH	7.300	7.288	0.032030	14	0.974900	8	8	0.789	0.772	1.045877	0.954337
NITRATE (mg/L)	5.838	5.275	0.598815	14	0.558862	8	8	1.776	1.976	1.238068	0.785333
PHOSPHATE (mg/L)	2.040	2.039	0.000806	14	0.999369	8	8	3.103	3.103	1.000159	0.999839
BOD (mg/L)	212.625	212.625	0.000000	14	1.000000	8	8	320.095	320.095	1.000000	1.000000
COD (mg/L)	2223.875	2223.750	0.000080	14	0.999937	8	8	3129.831	3129.895	1.000041	0.999958
CONDUCTIVITY (µs/cm)	392.250	392.250	0.000000	14	1.000000	8	8	385.951	385.951	1.000000	1.000000

Variable	SPECIES=*C. vulgaris*, EFFLUENT TYPE=Coffee T-tests; Grouping: DAY (Original Data) Group 1: Day10 Group 2: Day15										
	Mean	Mean	t-value	Df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	158.875	158.875	0.000000	14	1.000000	8	8	143.822	143.822	1.000000	1.000000
PH	7.175	7.175	0.000000	14	1.000000	8	8	0.755	0.755	1.000000	1.000000
NITRATE (mg/L)	5.688	5.500	0.173412	14	0.864810	8	8	2.267	2.053	1.219280	0.800343
PHOSPHATE (mg/L)	1.641	1.641	0.000000	14	1.000000	8	8	2.538	2.538	1.000000	1.000000
BOD (mg/L)	212.000	211.750	0.001560	14	0.998777	8	8	320.715	320.274	1.002755	0.997197
COD (mg/L)	2165.250	2165.250	0.000000	14	1.000000	8	8	3032.165	3031.914	1.000165	0.999832
CONDUCTIVITY (µs/cm)	238.125	238.000	0.001035	14	0.999189	8	8	241.633	241.578	1.000453	0.999539

Variable	SPECIES=*G. gelatinosa*, EFFLUENT TYPE=Coffee T-tests; Grouping: DAY (Original Data) Group 1: Day10 Group 2: Day15										
	Mean	Mean	t-value	Df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	306.875	306.875	0.000000	14	1.000000	8	8	314.601	314.601	1.000000	1.000000
PH	7.650	7.650	0.000000	14	1.000000	8	8	0.842	0.842	1.000000	1.000000
NITRATE (mg/L)	8.088	7.588	0.358456	14	0.725350	8	8	2.716	2.862	1.110404	0.893670
PHOSPHATE (mg/L)	2.383	2.382	0.000801	14	0.999372	8	8	3.120	3.121	1.000522	0.999469
BOD (mg/L)	213.000	213.000	0.000000	14	1.000000	8	8	319.860	319.860	1.000000	1.000000
COD (mg/L)	2267.250	2267.250	0.000000	14	1.000000	8	8	3203.949	3203.949	1.000000	1.000000
CONDUCTIVITY (µs/cm)	487.375	487.375	0.000000	14	1.000000	8	8	512.735	512.735	1.000000	1.000000

Variable	SPECIES=*S. salina*, EFFLUENT TYPE=Tea T-tests; Grouping: DAY (Original Data) Group 1: Day10 Group 2: Day15										
	Mean	Mean	t-value	Df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	131.938	131.938	0.000000	30	1.000000	16	16	90.0240	90.0240	1.000000	1.000000
PH	8.698	8.691	0.049319	30	0.960992	16	16	0.3920	0.3965	1.022872	0.965646
NITRATE (mg/L)	1.809	1.809	0.000000	30	1.000000	16	16	0.8853	0.8853	1.000000	1.000000
PHOSPHATE (mg/L)	30.208	30.050	0.034346	30	0.972828	16	16	12.9509	12.9894	1.005958	0.990973
BOD (mg/L)	482.437	482.437	0.000000	30	1.000000	16	16	329.0674	329.0674	1.000000	1.000000
COD (mg/L)	1124.625	1124.313	0.001009	30	0.999202	16	16	876.1023	875.8253	1.000633	0.999039
CONDUCTIVITY (µs/cm)	212.813	212.813	0.000000	30	1.000000	16	16	144.9359	144.9359	1.000000	1.000000

Variable	SPECIES=*C. vulgaris*, EFFLUENT TYPE=Tea T-tests; Grouping: DAY (Original Data) Group 1: Day10 Group 2: Day15										
	Mean	Mean	t-value	Df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	81.813	81.8125	0.000000	30	1.000000	16	16	31.9525	31.9525	1.0000	1.000000
PH	8.642	8.6394	0.024554	30	0.980573	16	16	0.3604	0.3596	1.0043	0.993428
NITRATE (mg/L)	4.819	1.4388	1.026222	30	0.312991	16	16	13.1446	0.9229	202.8646	0.000000
PHOSPHATE (mg/L)	28.870	28.6125	0.059126	30	0.953244	16	16	12.3435	12.2926	1.0083	0.987439
BOD (mg/L)	443.750	443.7500	0.000000	30	1.000000	16	16	320.4654	320.4654	1.0000	1.000000
COD (mg/L)	1005.063	988.8125	0.054215	30	0.957124	16	16	858.3473	837.0724	1.0515	0.923843
CONDUCTIVITY (µs/cm)	131.750	131.7500	0.000000	30	1.000000	16	16	51.8080	51.8080	1.0000	1.000000

Variable	SPECIES=*G. gelatinosa*, EFFLUENT TYPE=Tea T-tests; Grouping: DAY (Original Data) Group 1: Day10 Group 2: Day15										
	Mean	Mean	t-value	Df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	174.000	174.000	0.000000	30	1.000000	16	16	115.0559	115.0559	1.000000	1.000000
PH	8.731	8.716	0.103707	30	0.918092	16	16	0.4328	0.4194	1.064724	0.904932
NITRATE (mg/L)	2.426	2.361	0.146123	30	0.884802	16	16	1.2797	1.2607	1.030396	0.954525
PHOSPHATE (mg/L)	32.754	32.656	0.020702	30	0.983621	16	16	13.3388	13.4743	1.020428	0.969278
BOD (mg/L)	502.125	502.063	0.000565	30	0.999553	16	16	312.7137	312.7275	1.000088	0.999866
COD (mg/L)	1219.375	1219.000	0.001086	30	0.999141	16	16	976.5864	976.6913	1.000215	0.999673
CONDUCTIVITY (µs/cm)	280.500	280.500	0.000000	30	1.000000	16	16	185.3307	185.3307	1.000000	1.000000

Variable	SPECIES=*S. salina*, EFFLUENT TYPE=Sugar T-tests; Grouping: DAY (Original Data) Group 1: Day10 Group 2: Day15										
	Mean	Mean	t-value	Df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	177.000	177.000	0.000000	2	1.000000	2	2	11.3137	11.3137	1.000000	1.000000
PH	8.150	8.150	0.000000	2	1.000000	2	2	0.2121	0.2121	1.000000	1.000000
NITRATE (mg/L)	4.500	4.250	0.200000	2	0.859972	2	2	1.4142	1.0607	1.777778	0.819331
PHOSPHATE (mg/L)	1.550	1.500	0.074329	2	0.947514	2	2	0.6364	0.7071	1.234568	0.933049
BOD (mg/L)	420.000	420.000	0.000000	2	1.000000	2	2	113.1371	113.1371	1.000000	1.000000
COD (mg/L)	1250.000	1250.000	0.000000	2	1.000000	2	2	339.4113	339.4113	1.000000	1.000000
CONDUCTIVITY (µs/cm)	285.500	285.500	0.000000	2	1.000000	2	2	17.6777	17.6777	1.000000	1.000000

Variable	SPECIES=*C. vulgaris*, EFFLUENT TYPE=Sugar T-tests; Grouping: DAY (Original Data) Group 1: Day10 Group 2: Day15										
	Mean	Mean	t-value	df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	121.5000	121.5000	0.00	2	1.000000	2	2	4.94975	4.94975	1.000000	1.000000
PH	8.3500	8.3500	0.00	2	1.000000	2	2	0.07071	0.07071	1.000000	1.000000
NITRATE (mg/L)	4.5000	4.5000	0.00	2	1.000000	2	2	0.70711	0.70711	1.000000	1.000000
PHOSPHATE (mg/L)	1.3500	1.3500	0.00	2	1.000000	2	2	0.77782	0.77782	1.000000	1.000000
BOD (mg/L)	195.0000	195.0000	0.00	2	1.000000	2	2	7.07107	7.07107	1.000000	1.000000
COD (mg/L)	613.0000	613.0000	0.00	2	1.000000	2	2	69.29646	69.29646	1.000000	1.000000
CONDUCTIVITY (µs/cm)	196.0000	196.0000	0.00	2	1.000000	2	2	7.07107	7.07107	1.000000	1.000000

Variable	SPECIES=*G. gelatinosa*, EFFLUENT TYPE=Sugar T-tests; Grouping: DAY (Original Data) Group 1: Day10 Group 2: Day15										
	Mean	Mean	t-value	Df	p	Valid N	Valid N	Std.Dev.	Std.Dev.	F-ratio	P
TDS (mg/L)	196.000	196.000	0.000000	2	1.000000	2	2	1.4142	1.4142	1.000000	1.000000
PH	8.400	8.350	0.128037	2	0.909833	2	2	0.4243	0.3536	1.440000	0.884568
NITRATE (mg/L)	6.500	6.500	0.000000	2	1.000000	2	2	0.7071	0.7071	1.000000	1.000000
PHOSPHATE (mg/L)	2.350	2.350	0.000000	2	1.000000	2	2	0.4950	0.4950	1.000000	1.000000
BOD (mg/L)	543.000	543.000	0.000000	2	1.000000	2	2	165.4630	165.4630	1.000000	1.000000
COD (mg/L)	1724.500	1724.500	0.000000	2	1.000000	2	2	649.8311	649.8311	1.000000	1.000000
CONDUCTIVITY (µs/cm)	315.500	315.500	0.000000	2d	1.000000	2	2	2.1213	2.1213	1.000000	1.000000

APPENDIX XI: INFLUENT AND EFFLUENT OF PARAMETERS FROM DIFFERENT SITES

	COFFEE															
SITE	C01		C02		C03		C04		C05		C06		C07		C08i	
PARAMETER	IN	EF	IN	EF	IN	EF	IN	EF	IN	EF	IN	EF	IN	EF	IN	EF
TDS	227	230	801	798	1980	1980	1374	1380	344	356	309	309	344	344	309	295
PH	7.4	7.5	7.5	7.6	5.2	5.6	7.7	7.7	5.3	5.5	6.8	6.8	5.3	5.3	6.8	6.9
Nitrate	19	19	6.5	6	3	2	7	6	12	12	5.5	5.5	21	21	25	23
Phosphate	1.4	1.5	0.4	0.3	0.05	0.05	1.5	1	4	3	5	4	8	6	15	12
BOD	36	30	20	20	889	886	527	520	74	74	42	42	74	74	42	42
COD	140	130	3405	3410	16010	16000	4535	4535	1388	1388	403	403	1388	1370	403	400
Conductivity	379	384	1335	1330	3300	3300	2290	2300	574	580	515	515	574	574	515	492

	SUGAR				TEA					
SITE	S01		S02		T01		T02		T03	
PARAMETER	IN	EF	IN	EF	IN	EF	IN	EF	IN	EF
TDS	592	447	5.9	6.1	354	330	649	642	167	120
PH	6.7	7	40	30	6.98	7.1	7.25	7.5	7.77	8.2
Nitrate	30	26	7.2	5.9	2.5	2	4	3	0.2	0.1
Phosphate	4.8	4.1	40	34	85	80	70.6	66	4.58	3
BOD	643	500	4350	3784	880	760	692	660	300	71
COD	6547	5960	915	785	3320	3100	1744	1650	183	30
Conductivity	986	745	9.8	10	590	550	1083	1071	279	200

	TEA CONTINUED															
SITE	**T04**		**T05**		**T06**		**T07**		**T08**		**T09**		**T10**		**T11**	
PARAMETER	**IN**	**EF**	**IN**	**EF**	**IN**	**EF**	**IN**	**EF**	**IN**	**EF**	**IN**	**EF**	**IN**	**EF**	**IN**	**EF**
TDS	366	341	672	664	173	124	537	537	496	486	930	929	381	366	876	672
PH	6.98	7.1	7.25	7.5	7.77	8.2	7.2	7.5	7.18	7.4	8.5	10.7	6.8	7	7	7.3
Nitrate	2.5	2	4	3	0.2	0.1	4.8	4.5	2	1.5	5.5	5.3	3	2.5	6	4
Phosphate	85	80	70.6	66	4.58	3	90	88	48.1	45	99	97	90	85	75	71
BOD	880	760	692	660	300	71	150	150	378	360	790	770	1000	880	812	692
COD	3320	3100	1744	1650	183	30	1650	1650	1257	1235	2700	2620	3500	3320	2115	1744
Conductivity	590	550	1083	1071	279	200	866	866	800	784	1500	1498	614	590	1413	1083

SITE	**T12**		**T13**		**T14**		**T15**		**T16**		**T17**		**T18**		**T19**	
PARAMETER	**IN**	**EF**	**IN**	**EF**	**IN**	**EF**	**IN**	**EF**	**IN**	**EF**	**IN**	**EF**	**IN**	**EF**	**IN**	**EF**
TDS	193	173	568	537	818	496	951	929	435	366	709	672	202	173	651	537
PH	6.8	7.77	6.9	7.2	6.8	7.18	9.2	10.65	6.5	6.98	6.8	7.25	6.8	7.77	6.9	7.3
Nitrate	11	10	5.9	4.8	4.2	3	6	5.3	3	2.5	6	4	8	6	6.8	5
Phosphate	64	58	101	90	60	47	74	69	93	85	78	70.6	65	54	95	82
BOD	120	71	268	150	690	400	980	770	1100	880	876	692	200	71	200	150
COD	347	183	2218	1650	1500	1257	3406	2620	3640	3320	1912	1744	465	183	1800	1650
Conductivity	312	279	916	866	1320	800	1533	1498	702	590	1144	1083	325	279	1050	866