CURRENT STUDIES IN COMPARATIVE EDUCATION, SCIENCE AND TECHNOLOGY, VOLUMES 5&6, NUMBERS 1-2, 2019, PP. 122-132

Bioremediation Potentials of Two Cyanobacterial Isolates on Rivers Polluted with Abattoir Effluent

WILLIAMS, JANET OLUFUNMILAYO & CHIBUIKE, PRINCESS MEBOM
Rivers State University, Port Harcourt, Nigeria

ABSTRACT Discharge of untreated domestic and industrial wastewater into water bodies is posing a serious eutrophicaton threat, leading to a slow degradation of water resources. A number of physical, chemical and biological methods have been developed for the treatment of wastewater; among these, the use of cyanobacteria is considered a more ecofriendly and economical approach. Cyanobacteria are versatile phototrophic aquatic microbes that perform multiple roles in the environment. The Cyanobacterial species investigated in this study were highly beneficial in the remediation of three Rivers (Ogbunuabali, Rumuokoro and Diobu) polluted with abattoir effluent. The Cyanobacteria isolated were identified as *Anabaena* and *Gloeocapsa* species using the river water collected at the point of discharge for each Abattoir in Port Harcourt, Rivers State. The physicochemical parameters (Temperature and pH) and nutrients (phosphate and nitrate) in the various environments, Ogbunuabali (AN_1, GP_1 and AN_1+ GP_1), Rumuokoro (AN_2, GP_2, AN_2+ GP_2) and Diobu (AN_3, GP_3 and AN_3+ GP_3) river water samples enhanced the growth of the cyanobacterial isolates.The microbiological analysis showed that the cyanobacterial counts were higher in the mixed cultures (AN_1+ GP_1 (9.70 x 10^5 cfu/ml), AN_2+ GP_2 (9.50 x 10^5cfu/ml) and AN_3+ GP_3 (9.37 x 10^5cfu/ml)) than the single cultures especially on day 7 which could be due to the presence of a consortium of organisms and/or nutrients (Nitrogen and Phosphorus). The effectiveness of the Cyanobacterial species used in this analysis for remediating the river water for each site was in the following descending order: Ogbunuabali (AN_1+ GP_1 > GP_1> AN_1), Rumuokoro (AN_2+ GP_2 > GP_2>AN_1) and Diobu (AN_3+ GP_3> GP_3> AN_3). Statistically, the mixed cultures from the three different sites (AN_1+ GP_1, AN_2+ GP_2 and AN_3+ GP_3) were more effective and capable of remediating rivers polluted with abattoir effluent than the single cultures. The analysis of variance (ANOVA) showed that at 95% and 99% confident levels, there were significant differences among the samples and they varied in their ability to remediate the environment.

Keywords: Anabaena spp, *Gloeocapsa* spp, Abattoir effluent, single and mixed cultures

Introduction

In many parts of the world, human activities such as animal production still impact negatively on the environment and biodiversity. The consequences of man-made pollution amongst a lot include transmission of diseases by water borne pathogens, eutrophication of natural water bodies, accumulation of toxic or recalcitrant chemicals in the soil, destabilization of ecological balance and negative effects on human health (Boadi and Kuitunen, 2003; Amisu *et al*., 2003). Meat consumption and production for the ever increasing World population has some pollution problems associated with it (Hinton *et al*., 2000). In most countries, meat pollution arises as a re-

sult of failure in adhering to Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP) (Amisu *et al*., 2003). Deliberation is hardly given to safety practices during animal transport,slaughter and dressing in the abattoir. For example, during dressing, the oesophagus of cattle and sheep should be sealed to prevent leakage of animal contents. These unsafe practices often lead to contamination of hides, hooves and content of alimentary tract during slaughtering and negatively impact the environment, including microbes in the soil as well as surface and ground water (Amisu *et al*., 2003).

Phytoremediation, is the use of green plants (including Cyanobacteria or lower plants) and associated microflora for the removal or degradation of pollutants including nutrients and heavy metals from polluted water bodies which seems to be a promising option (Ali *et al.,* 2013; Franchin*o et al*., 2013; Richards and Mullins, 2013). In 1955, Oswald and Gotaas led researches on the possibility of using cyanobacteria for water treatment. In 1957, Oswald *et al.,* reported designs of innate treatment systems driven by solar energy, making wastewater treatment inexpensive and sustainable.

Bioremediation means the use of microorganisms to clean-up a polluted or contaminated site. The prefix "bio" depicts the process as carried out by living organisms while "remediation" shows that the process results to the cleaning of a polluted environment (Sunderamoorthy *et al*., 2001). The process makes use of microorganisms to catalyze the breakdown of harmful substances to useful forms. Since microbes are used, the procedure is cheap and is used to proficiently reduce the threat of environmental pollution. The liquid wastes from populated and industrial areas results to a key source of water pollution. These industrial effluents are mostly emptied into open drains which eventually join open water bodies (Kumari *et al*., 2006). Novel machineries are being planned to access the treatment of waste water and the utilization of Cyanobacteria is one of them (Ash and Jenkins, 2006). Cyanobacteria represent an extensive category of organisms made up of single cells which are prevalent in fresh and sea environments. Cyanobacteria seldom persist in symbiotic relationship with other life forms such as cycads and fungi and provide valuable food and nutrient for many other organisms (Burch *et al.,* 2001). Some species can fix atmospheric nitrogen. Cyanobacteria are very vulnerable to abrupt physical and chemical changes of light, salinity, temperature and nutrient composition (Semyalo, 2009). Phytoremediation showed massive prospective in wastewater and industrial runoff treatment, remediation of aquatic and terrestrial habitats, chemical industries, bio-fertilizers, food, feed, fuel, etc. More so, pH, carbon dioxide, organic matter, alkalinity, nitrates and phosphates are factors important in determining the distribution of cyanobacteria (Podda *et al.,* 2000).

Wastewater is generally loaded with contaminants in the form of nutrients, heavy metals, hydrocarbons etc. The existence of nutrients especially nitrogen (N) and phosphorus (P), in the form of nitrate, nitrite, ammonia/ammonium or phosphorus in open water bodies leads to eutrophication (Liu *et al.,* 2010; Yang *et al.,* 2008). Cyanobacteria are usually present in wastewaters and can play a role in the self-purification of these waters. The aim of this study was to identify the bioremediation potentials of two cyanobacterial species (*Anabaena* and *Gloeocapsa spp.*) in Rivers (Ogbunuabali, Rumuokoro and Diobu) polluted with abattoir effluent.

Materials and Methods

Study Areas

The study areas used were Abattoirs located at Ogbunuabali, Rumuokoro and Diobu in Port Harcourt, Rivers State. According to the butchers in the abattoirs, 5 cows or more are killed every Saturday; hence, they are fairly busy slaughter houses.

Sample Collection

River water was collected from the points of discharge into the rivers where the concentration of the abattoir effluent was high at Ogbunuabali, Rumuokoro and Diobu Rivers using sterile plastic bottles. The effluent from the abattoirs was also collected using sterile plastic bottles. The samples were then taken to the Microbiology laboratory in Rivers State University, Port Harcourt for analysis.

Bacteriological analyses

Single cell technique (Williams and Youngtor, 2017) was used for the isolation of the cyanobacteria in each sample area. Using 1ml of sterile Pasteur pipette, an aliquot of each sample was placed on a clean glass slide, inserted on the microscope, covered with cover slip and viewed under x10 magnification of the microscope. The process was continued until the cyanobacterial species were properly identified by their morphological characteristics. Ogbunuabali slaughter river water contained *Anabaena* and *Gloeocapsa* species, Rumuokoro slaughter river water contained *Nostoc*, *Gloeocapsa* and *Anabaena* while Diobu slaughter river water contained mostly *Anabaena* and *Oscillatoria*. The isolates from the Ogbunuabali abattoir was however used for the bioremediation of the three sites.

Media Used

Nutrient Agar

This was prepared according to manufacturer's instruction of dispensing 28g of nutrient agar into 1000ml of distilled water. Mass/Volume relationship was used to compute actual required measurements. The mixture was mixed vigorously and then sterilized by autoclaving at121 ◦C at 15psi for 15minutes. Antibiotics (Chloramphenicol) and Nystatin (an anti-fungal agent) were mixed in the media and allowed to cool for about 10seconds before pouring into the petri dishes. Chloramphenicol was added to inhibit bacterial growth while Nystatin was added to inhibit fungal growth. The media was allowed to cool and solidify and dried in a hot air oven before inoculation with the river water (aqua culture).

Blue Green Media (B.G. 11)

BG 11, a modified broth medium was compounded and used for growth optimization of the cyanobacteria. BG 11 consists of two solutions (A and B) and micronutrient solution. Solutions A and B, containing (in grams): A; $NaHCO_3$ 11.61 g, Na2CO_3 3.53 g, K_2HPO_4 0.5 g dissolved in 500 ml distilled water and B; $NaNO_3$ 5 g, K_2SO_4 1 g, NaCl 1 g, $MgSO_4.7H_2O$ 0.2 g, $CaCl_2$ 0.04 g, and 1 ml EDTA (0.5 M). Micronutrient solution consisted of the following trace metals (in milligrams) dissolved in one liter distilled water: Na_2-EDTA 50 g, H_3BO_3 618 g, $CuSO_4.5H_2O$ 19.6 g, $ZnSO_4.7H_2O$ 44 g, $CaCl_2.6H_2O$ 20 g, $MnCl_2$ 12 g and $Na_2MoO_4.2H_2O$ 12.6 g. Solutions A and B were sterilized by autoclaving separately at 121°C at 15 psi for 15 minutes. Micronutrient solution was sterilized by filtration through Whatman's filter paper to avoid interaction and precipitation of heavy metals. After sterilization, solutions A and B were combined and 1 ml of the micronutrient solution and were added. Optimization of cyanobacterial growth included adjusting the light/dark cycle,

with 16/8 h of white light, temperature at 25 to 30°C, and shaking of the cultures thrice daily, all of which led to enhancement of mass production. Prior to biodegradation bioassays, all cultures were tested for the presence of heterotrophic bacteria microscopically and by plating on bacterial nutrient medium (nutrient agar) and incubating at 30°C for 1 week. Only axenic cultures, either uni- or multi-algal species, were used in the assays.

Inoculation

About 1ml of river water from each site was dispensed into 9ml of normal saline. An aliquot of the sample was transferred using a sterile 1ml pipette unto the agar plate which was incubated for 7days with 16/8 h of white light, at a temperature of 25 to 30°C. The organisms were then viewed and identified microscopically before being transferred to a mixture of the river water, abattoir effluent and Blue Green Media (B.G.11) for growth monitoring and optimization.

Growth Monitoring

The pure cultures of *Anabaena* and *Gloeocapsa* species were obtained and transferred by using sterile wire loop into test tubes containing normal saline until it was turbid. About 5ml of sample was taken by using a sterile 10ml pipette and emptied into nine conical flasks containing the river water from each site (three for each site). Mixed cultures of *Anabaena* and *Gloeocapsa* were set up as well as single cultures in the polluted river water environment.

Growth was monitored to determine the stimulatory or inhibitory effects of the contents of each of the pollutants on the test cyanobacterial species in order to determine if the species could remediate the pollutant and to identify the most promising bioremediation species.

The B.G.11 and abattoir effluent (which served as growth nutrient) were emptied into nine (9) conical flasks containing 250ml of the abattoir wastewater and labeled, AN_1-AN_3 for *Anabaena*, GP_1-GP_3 for *Gloeocapsa* and AN_1+GP_1-AN_3+GP_3 for the mixed culture holding conical flasks. Appropriate controls were also set up containing only *Anabaena* (Control AN) and *Gloeocapsa* species (Control GP).

After the sample preparation, the flasks were bloomed for 7days under 16/8 h of white light, and a temperature between 25 to 30°C. The conical flasks were intermittently shaken three times daily to enhance growth by preventing sedimentation of the test organisms and avoiding thermal stratification which involves gas exchange between the culture medium and air to ensure that cells of the population received equal amount of light and nutrients.

Determination of Cyanobacterial Concentration (Optical Density)

From the conical flasks labeled AN_1-AN_3 for *Anabaena*, GP_1-GP_3 for *Gloeocapsa*, mixed cultures and the control , optical densities were determined on Days 0, 3 and 7 of the experiment. On day zero (0), about 5ml of sample was transferred into a test tube containing 9ml of normal saline using a sterile pipette. The sample was serially diluted to decrease turbidity. 1ml of the sample was then transferred into a polystyrene cuvette, 1ml of sterile BG11 served as the blank. The UV-spectrophotometer was set at 600nm and the optical densities were read. This procedure was repeated thrice on days 3 and 7 of the experiment cycle.

Physicochemical Parameters of the Samples

The physicochemical parameters were measured using standard analytical procedures (AOAC, 2000). The pH meter used was pocket-sized HANA pHep + HI 98108 with automatic temperature compensation. Total organic carbon was determined by

dichromate wet oxidation method of Walkley and Black as modified by Dhyan *et al*., (1999). Nitrate content was determined using the macro Kjeldahl digestion method of Brady and Weil (1999) and available phosphorus was determined using the method reported by Olsen and Sommers (1982). Sulphate was determined using the turbidometric method. Standard methods were used for the determination of Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) (AOAC,2000).

Total Cyanobacterial Counts

The average total cyanobacterial population present in each sample at the beginning of the experiment (day 0) to the end (day 7) were estimated using nutrient agar. Inoculated petri dishes were incubated at inverted positions for24hours at 37◦C, after which plates were checked, colonies counted and results obtained.

Statistical Analysis

The analysis of variance as described by Nduka and Ogolime (2000) was used to ascertain the significant difference, at 95% confidence interval, between the cyanobacteria total viable count of the different samples.

Results

The optical densities of the three locations (Ogbunuabali, Rumukoro and Diobu) containing the cyanobacterial species on day 7 is presented on fig. 1 below with the mixed cultures having the highest absorbance.

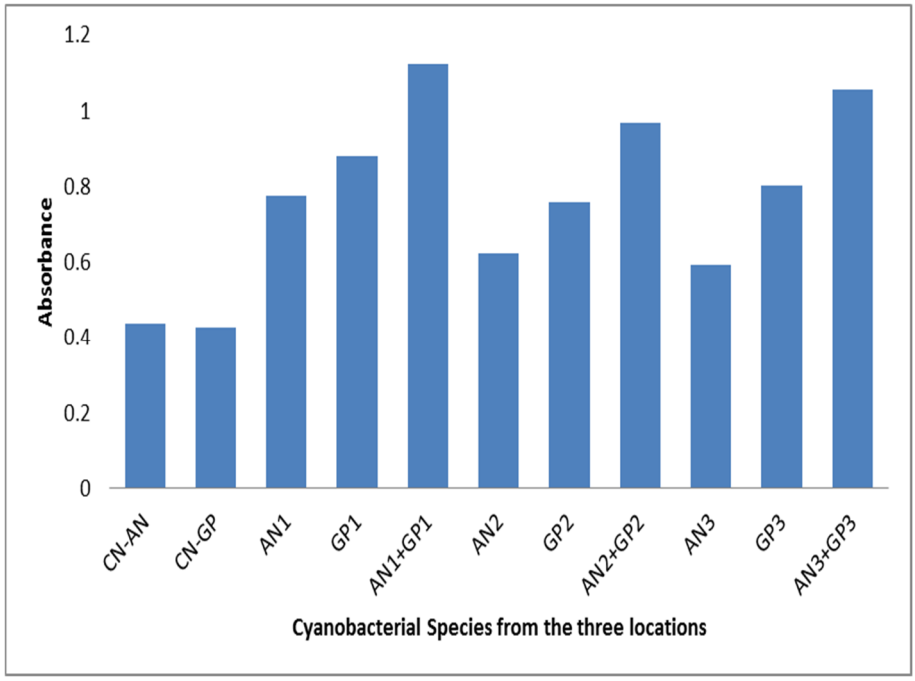

Fig 1:Optical densities of the different samples on Day 7

KEY:
AN_1, GP_1 and AN_1+GP_1: Samples from Ogbunuabali river water containing *Anabaena*, *Gloeocapsa* and a mixture of *Anabaena* and *Gloeocapsa species.*
AN_2, GP_2 and AN_2+GP_2: Samples from Rumuokoro river water containing *Anabaena*, *Gloeocapsa* and a mixture of *Anabaena* and *Gloeocapsa species.*
AN_3, GP_3 and AN_3+GP_3: Samples from Diobu river water containing *Anabaena*, *Gloeocapsa* and a mixture of *Anabaena* and *Gloeocapsa species.*
C-AN: Control containing *Anabaena species* only
C-GP: Control containing *Gloeocapsa species* only

The table below shows the total cyanobacterial count from day 0 to 7 for the three sites.

Table 1: Total Cyanobacterial Counts

	Day (cfu/ml)		
Sample	**0**	**3**	**7**
CN-AN	1.85×10^5	2.10×10^5	2.22×10^5
CN-GP	1.84×10^5	2.11×10^5	2.22×10^5
Ogbunuabali			
AN_1	3.93×10^5	4.09×10^5	4.17×10^5
GP_1	3.83×10^5	3.96×10^5	4.80×10^5
AN_1+GP_1	7.56×10^5	8.26×10^5	9.70×10^5
Rumuokoro			
AN_2	4.10×10^5	4.23×10^5	4.46×10^5
GP_2	3.84×10^5	4.06×10^5	4.12×10^5
AN_2 +GP_2	8.09×10^5	8.47×10^5	9.5×10^5
Emenike			
AN_3	4.00×10^5	4.15×10^5	4.43×10^5
GP_3	4.11×10^5	4.43×10^5	4.63×10^5
AN_3 +GP_3	8.35×10^5	9.19×10^5	9.37×10^5

KEY:
AN_1, GP_1 and AN_1+GP_1: Samples from Ogbunuabali river water containing *Anabaena*, *Gloeocapsa* and a mixture of *Anabaena* and *Gloeocapsa species.*
AN_2, GP_2 and AN_2+GP_2: Samples from Rumuokoro river water containing *Anabaena*, *Gloeocapsa* and a mixture of *Anabaena* and *Gloeocapsa species.*
AN_3, GP_3 and AN_3+GP_3: Samples from Diobu river water containing *Anabaena*, *Gloeocapsa* and a mixture of *Anabaena* and *Gloeocapsa species.*
C-AN: Control containing *Anabaena species* only
C-GP: Control containing *Gloeocapsa species* only
From table 1, the microbiological analysis showed that the total cyanobacterial counts were higher in the mixed cultures (AN_1+GP_1, AN_2+GP_2 and AN_3+GP_3) than the single cultures, especially on day 7 which was the last day of the analysis. The higher counts could be as a result of the mixed culture (*Anabaena* and *Gloeocapsa*) or presence of nutrients (Nitrogen and Phosphorous) that favoured the cyanobaterial growth (Williams and Youngtor,2017 ; Ekundayo *et al.,* 2010).
Physicochemical parameters of abattoir effluent and river water are shown on table 2 below.

Table 2: Physicochemical parameters of Abattoir effluent and river water from sites

	Abattoir effluent	River water		
Parameters	A	A	B	C
Nitrate (mg/l)	5.56	5.14	5.22	5.19
Temperature (^{0}C)	27.6	28.1	26	27
Phosphate (mg/l)	0.073	0.070	0.073	0.070
Sulphate (mg/l)	1.0	1.2	1.2	1.25
pH	7.2	7.7	7.5	7.3
Total organic carbon (mg/l)	0.17	0.13	0.13	0.14
BOD(mg/l)	42	36	35	36
COD (mg/l)	275	254	255	254
DO (mg/l)	1.1	1.0	1.1	1.0

KEY:
River water A= Ogbunuabali river water
River water B= Rumuokoro river water
River water C= Diobu river water

The abattoir effluent and river water contain essential nutrients required for the growth of the test organisms and they include phosphate (0.070-0.073 mg/l), sulphate (1.00-1.25 mg/l), nitrate (5.14-5.56mg/l). Other physicochemical parameters that supported their growth were temperature (26 -28.1 ^{0}C), pH (7.2-7.7) and Total Organic Carbon (0.13-0.17 mg/l) (AOAC, 2000).Temperature of samples in ogbunuabali river water (*Anabaena*(AN_1) 28^{0}C, *Gloeocapsa* (GP_1) 27^{0}C, *Anabaena*+*Gloeocapsa* (AN_1+GP_1) 28.2^{0}C), Rumuokoro river water (*Anabaena*(AN_2) 27^{0}C, *Gloeocapsa* (GP_2) 27^{0}C, .*Anabaena*+*Gloeocapsa* (AN_2+GP_2) 28^{0}C) and Diobu river water (*Anabaena*(AN_3) 27^{0}C, *Gloeocapsa* (GP_3) 26^{0}C, *Anabaena*+*Gloeocapsa* (AN_3+GP_3) 28^{0}C).

Discussion

The mixed cultures remediating the three sites (AN_1+GP_1, AN_2+GP_2 and AN_3+GP_3) had the highest absorbance due to the presence of higher amounts of chlorophyll, elevated concentration of oxidizable organic matter and trace dissolved oxygen, nutrients (nitrogen and phosphorous) in the polluted rivers investigated and some other physical parameters (pH and temperature) could have enhanced the growth of the Cyanobacteria (AOAC, 2000; Williams and Youngtor, 2017). Higher total cyanobacterial counts observed in the mixed cultures (AN_1+GP_1, AN_2+GP_2 and AN_3+GP_3) than the single cultures, especially on day 7 could be as a result of the consortium (*Anabaena* and *Gloeocapsa*) or presence of nutrients (Nitrogen and Phosphorous) that favoured the cyanobacterial growth (Williams and Youngtor,2017 ; Ekundayo *et al.,* 2010).

The physicochemical parameters of the abattoir effluent and the river water from the different sites supported the growth of the organisms. The observed pH from the Rivers was within permissible limit (WHO, 2004). The temperature of the Rivers observed was within permissible limits (WHO, 2004). The increased temperature of the mixed cultures must have been due to increased oxygen depletion (reduction in dissolved oxygen) in the polluted environment (Williams and Youngtor, 2017 ; Ekundayo *et al.,* 2010). Elevated BOD, COD, phosphates and ni-

trates values as well as extremely low DO favored the growth of Cyanobacteria more than any other algae (Venkateswarlu, 1976). This concurred with the studies of Williams and Youngtor (2017), Haande (2008) and Jeganathan (2006) in different industrial waste water.

In this work, the abattoir effluent and river water showed a considerable quantity of nitrate and phosphate with high levels of BOD and COD and exceedingly low DO level (Williams and Youngtor, 2017 ; Larsson *et al*., 2009).

The native Cyanobacteria from abbatoir effluent and river water were found to enhance the degradation of the pollutant. They were highly efficient in the remediation of the three polluted river water samples used in this study.

The analysis of variance (ANOVA) of the total cyanobacterial count in the samples from the three sites showed that the mixed cultures (AN_1+GP_1, AN_2+GP_2 and AN_3+GP_3) had the highest counts. The effectiveness of the cyanobacterial species used in this analysis for remediating the river water from the three different sites were in the following descending order for each site; Ogbunuabali (AN_1+GP_1 > GP_1 > AN_1), Rumuokoro (AN_2+GP_2 >AN_2 > GP_2) and Diobu (AN_3+GP_3 > GP_3 > AN_3). The mixed cultures from the three different sites (AN_1+GP_1, AN_2+GP_2 and AN_3+GP_3) were more effective in remediating the river water polluted with abattoir effluent than the single cultures.

Conclusion

This work revealed that Abattoir effluent and Blue-Green (B.G. 11) medium are sources of essential nutrients and growth media for the cultivation of cyanobacteria. The study also revealed that the cyanobacterial species studied showed high resistance to the toxicity of the pollutant (abattoir effluent in the river water), they rather utilized the nutrients in the pollutant to grow. These findings are very important regarding the practical use of *Anabaena* and *Gloeocapsa* species in large scale. It also showed that the effectiveness of these organisms in phytoremediation vary when used as single cultures and in consortium.

Correspondence
Williams Janet Olufunmilayo
Department Of Microbiology
Rivers State University, Port Harcourt
Nigeria
Email: funmikemwilliams@ymail.com; janet.williams@ust.edu.ng

References

Ali, H., Khan, E., Sajad, M. A. (2013). Phytoremediation of heavy metals—concepts and applications, *Chemosphere* 91:869–881.

Amisu, K. O., Coker, A. O., On, S. L. W., Isokpehi, R. D. (2003). *Arcobacter butzlieri* strains from
poultry abattoir effluent in Nigeria. *Medical Journal* 80: 218-221.

Ash N, J. M (2006). Biodiversity and poverty reduction: the importance of biodiversity for ecosystem services. Final report prepared by the United Nations Environment Programme World Conservation Monitoring Centre (UNEP-WCMC) (http://www.unepwcmc.org) for the Department for International Development (DFID)

AOAC, (2000). Method of analysis 14th edition, Association of Official analytical Chemists, Arignton.
6: 503-515.

Boadi, K. O., Kuitunen, M. (2003). Municipal solid waste management in the Accra metropolitan area, Ghana. *The Environmentalist* 23: 211-218.

Brady, N.C. and Weil, R.R. (1999) The nature and properties of soils. 12th edition . Upper Saddle River, NJ: Prentice Hall, inc. 881

Burch, M. D., Chow, C. W. K., Hobson, P. (2001). *Algaecides* for control of toxic cyanobacteria Proceedings of the 2001 water Quality Conference, American Water Works Association, Nashville Tennessee, 1:1-15.

Dhyan,S., Chhonkar, P.K. and Pandey, R.N. (1999) Soil, Plant and water analysis - A method manual. Indian Agricultural Research Institute, New Delhi.

Ekundayo, E.O, Emede, T.O and Osayande, D.I (2010). Effects of crude oil spillage on growth and yield of maize (zea mays L.) in soils mid-western Nigeria. Plants, food and Human Nutrition, 56: 313-224.

Franchino, M., Comino, E., Bona, F., Riggio, V. A. (2013) Growth of three cyanobacteria strains and nutrient removal from an agro-zootechnical digestate. *Chemosphere* 92:738–744.

Haande, S., Rohrlack, and T., Semyalo, R. P. (2010). Phytoplankton dynamics and cyanobacterial dominance in Murchison Bay of Lake Victoria (Uganda) in relation to environmental conditions. 98: 901-924.

Hinton, M. H., Mead, G. C., Rowlings, C. (2000). Microbiology control in the meat industry. *Flair flow Europe technical manual* 2: 4-12.

Jeganathan, K. (2006). Bioremediation studies on oil refinery industry effluent using *Oscillatoria earli* Gartner. M.Phil. dissertation. Bharathidasan University. Tiruchirapalli.

Kumari, S. B., Kirubavathy, A. K. and Thirumalnesan, R. (2006). Stability and water quality criteria of open drainage municipal sewage water at Coimbatore used for irrigation. *Journal of Environmental Biology* 27(4): 709-712.

Larsson P., Kizito, Y.S., Miyingo,K. A., Luyiga, S., Haande, S., Semyalo, R.P., Asio, S.M., Odong, R., Lyche,S.A., Brettum, P. (2009). Development in Murchison Bay. In: Larsson P, Kirumira E, Steigen AL, Miyingo-KA (Eds.). Sharing Water: Problems, Conflicts and Possible Solutions-the Case of Kampala. Universities for laget, Oslo. 221-230.

Liu, W., Zhang, Q., Liu, G. (2010). Lake eutrophication associated with geographic location, lake, morphology and climate in China. *Hydrobiologia,* 644:289–299.

Nduka, E.C. and Ogolime,O. J.(2000) Statistics: Concepts and methods, Crystal Publishers, PH, Nigeria, 213

Olsen, S.R., Sommers, L.E. (1982) Determination of available phosphorus . In " Method of Soil Analysis", 2nd edition .American Society of Agronomy.

Podda, F., Zuddas, P., Minacci, A., Pepi, M., Balfi, F. (2000). Heavy metal coprecipitation with hydrozincite from mine waters caused by photosynthetic microorganisms. *Applied Environmental Microbiology* 66(11): 5092-5098.

Richards, R. G., Mullins, B. J. (2013) Using cyanobacteria for combined lipid production and heavy metal removal from leachate. *Ecological Modelling* 249:59–67.

Semyalo, R. P. (2009). The effects of cyanobacteria on the growth, survival, and behaviour of a tropical fish (*Nile Tilapia*) and zooplankton (*Daphnia lumholtzi*). Ph.D. Thesis, University of Bergen, Norway, 1:1-17

Sunderamoorthy, P., Kunchithapata, J., Thamizhiniyan, P. and Benkateslu, S. (2001). Effect of fertilizer effluent on germination and seedling growth of groundnut varieties. *Journal of Ecoboilogy* 13(1): 3-8.

Venkateshwarlu, V. (1976). Taxonomy and ecology of algae in the river Moosi. Hyderabad (India). Nova Hedwigia, 27: 661 -676.

Williams, J.O. and Youngtor, T.P. (2017) Remediation of pesticide-polluted river using *Anabaena* and *Nostoc* spp. *Journal of Environmental Science, Toxicology and Food Technology*,11(7): 71- 76.

World Health Organization, (2004). Guidelines for Drinking water Quality. *3rd* edition, Volume 1 Recommendations*,* 210-220.
Yang, X., Wu, X., Hao, H., He, Z. (2008). Mechanisms and assessment of water eutrophication. *Journal Of Zhejiang University of Science and Biotechnology* 9:197–209.