International Research Journal of Microbiology (IRJM) (ISSN: 2141-5463) Vol. 2(11) pp. 428-436, December 2011
Available online http://www.interesjournals.org/IRJM

Full Length Research Paper

An investigation of the distribution of microalgae within an oil production facility in the Niger Delta Region of Nigeria

Agwa, O.K, Ire, F.S. and Abu, G.O*

Department of Microbiology, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Nigeria

Accepted 30 November, 2011

A survey of the physico-chemical status and microalgae profile from the Obagi flare pit, within the Niger-Delta Region of Nigeria were carried out during the rainy and dry seasons. The result of the physico-chemical properties of the waste water samples showed different variations as follows: nitrate (2.65-8.855), calcium (0.675- 8.01), magnesium (1.21-2.41), ammonia (0.038- 0.090), phosphate (0.058- 0.54), total hardness (10.01- 24.0), temperature (27.2 - 31.6), Hydrogen-ion concentration (pH), (11.6 - 13.2), bicarbonate (930- 1900), salinity (2418.7- 7581.0) and sulphate (1.48 – 3.44). Lead, iron, manganese, vanadium, nickel, cadmium and chromium were among the heavy metals detected. There were up to six phyla of microalgae with about nineteen genera identified. Cyanophyta were the dominant microalgae with *Oscillatoria* (12.73%) predominant throughout the investigation while *Spirulina, Chlamydomonas, Cryptomonas, Gleotrichia, Cosmarium and Rhizochloris* at 1.82% were the least in occurrence. This site is a viable bioresource with potential in environmental biotechnology and in the study of remote sensing of microbial biomass.

Keywords: Heavy metals, microalgae, Niger delta, physicochemical properties, remote sensing waste water

INTRODUCTION

Microalgae are a diverse group of Prokaryotes with varying morphological and physiological properties, and have attracted increasing interests because of their immense potentials (Apt and Behrens, 1999; Lee, 1999; Cohen, 2000; Chisti *et al*., 2003; Olaizola, 2003, Tramper *et al*., 2003). Their common feature is their oxygenic photosynthesis, similar to that in higher plants, and they make large contributions to the equilibrium of the earth's atmosphere by producing oxygen and removing carbon (IV) oxide (Tandeau de marsac and Houmard, 1993).Vast interest in microorganisms obtained from extreme environment has grown considerably because they represent an innovative approach to obtain new underexplored resources (Romano *et al.,* 2000). In screening programs to obtain new prokaryotes from extreme environments, samples are collected from areas that showed high salinity and alkalinity. Alkaline-halophilic environments are rich in photosynthetic primary producers, resulting in dense populations of microalgae that support microbial community (Jones *et al*., 1998). Microalgae have close interactions with their environments, the parameters which define their environment such as temperature, light, nutrient concentrations and pH have to be at optimum level with their growth rate increasing directly proportional with light intensity at optimal conditions (Lee, 1999; Molina *et al.,* 1999).

The Niger Delta one of the World largest wetlands in the world, lies between latitudes 4-6^{0}N and longitude 5-8^{0}E with high biodiversity, characteristic swamps, waterways, vast flood plains, mangrove forest areas and fishing villages. Obagi is an onshore oilfield within the Niger Delta of Nigeria known for oil production and gas flaring (Guillonneau*, et al*.,2009). Flaring contributes a measurable percentage of the world's total emission of green house gases resulting in air pollution (Moffat and Linden, 1995). Treated wastewater from crude oil

*Corresponding authors E-mail: gideonabu1@yahoo.com; o_agwa@yahoo.com

production is discharged after oil-water separation process into concrete pits or ponds within the environment (DPR, 1991). While in the pit, the wastewater contaminates the soil environment as a result of overflow or seepage from faulty waste pits resulting in significant changes in the ecosystem. Obire (1988) reported the reduction in the number of microorganisms in polluted soils when compared with non polluted soil. Marshall and Devirry (1988) revealed an increase in the number of viable microorganisms during the hotter, drier month with a relatively smaller and less viable population during the cooler, wetter season. Obire and Wemedo (2002) assessed the effect of seasons on the population of bacteria and fungi in oilfield water polluted soil in the Niger delta area of Nigeria. There has not been information on the phytoplankton associated with the Obagi flare pit within the Niger Delta region of Nigeria and its significant economic importance. In this paper, we report the isolation and identification of the associated phytoplankton found in the wastewater and the ecological characteristics of the area. The site is also a potential site for the study of remote sensing of biomass.

MATERIALS AND METHODS

The study area

Obagi is an onshore oil field located on OML 58, 85 km north-west of Port-Harcourt, Nigeria as shown in Figure 1. It is composed of 26 stacked reservoir level with an estimated total OOIP of 1.2Gbbls. The Obagi onshore oil field was discovered in 1964, with oil production in 1966 and valorization of gas by Obite gas plant since 1999.The Obagi flare pit is a brackish water marsh located in Ogbogu Elf flow station (Obagi base), Ogbogu in Ogba-Egbema Ndoni Local Government Area (ONELGA), Rivers State, Nigeria. The flare pit was constructed into which crude oil wastewater were channeled to prevent flooding of the area during the rainy season and to avoid pollution of the environment. The edges of the pit were raised with mud and chemically treated sand to prevent spilled oil being carried by flood water during the rainy season. At the Southern end of the pit are two pipes projecting into the atmosphere and through which gas is being flared. The pit experience algal bloom all the year round because of the crude oil waste that provide nutrient for the algae, and artificial light providing warmth by the flaring gas. The climatic condition of the area is characterized by a dry season from December – March and a wet season from April – November with monthly rainfall of up to 30.60cm at a temperature range between 28 – 30^{o}C. The flare pit has the cordinate $5^{0}14^{l}16^{0}$N and $6^{0}37^{l}46^{0}$E.

Sample collection

Subsurface water samples were collected from a depth of 25 – 30cm from July to January thus covering the rainy and dry seasons. The samples were collected twice a month using sterile screw-capped containers and taken to the laboratory immediately for analysis. The concentrations of heavy metals were investigated every four months. Phytoplankton composition of the flare pit was carried out during the same period.

Determination of physico-chemical parameters and heavy metals

Nitrate, sulphate, phosphate, pH, temperature, carbonate and salinity were among the parameters determined according to standard methods of the American Public Health Association (1998). Hydrogen ion concentrations were carried out using an automatic digital pH meter (model Mettle Delta-340) made in England. Heavy metal concentrations were detected with SH INAD ZU-AA-6300 atomic absorption flame emission spectrophotometer (AAFES). The metals analysed for include Lead, Iron, Mercury, Nickel, Cadmium, Chromium, Manganese and Vanadium. Total hydrocarbon content was determined by gravimetric methods.

Isolation of Microalgae

The single cell isolation technique (Burris, 1977) was used for the isolation of the microalgae. Using 1ml of Pasteur pipette; an aliquot of the sample was placed in a watch glass and viewed under the X40 objective of the Hm-lux leitz light microscope. A micropore Pasteur pipette fitted with a rubber bulb was used to suck out cells of the microalgae, transferred into another watch glass containing 1ml of normal saline to obtain a unialgal culture of the microalgae and the process was continued until the microalgae were identified by their characteristic morphologies.

RESULTS

Investigation of the study area was carried out between the months of July to January. Figures 2- 6 represent the physico-chemical parameters of the water sample. Highlights includes,mg/l Nitrate, 2.65-8.855; Calcium, 0.675- 8.01; Magnesium, 1.21-2.41; Ammonia, 0.038-0.09; Phosphate, 0.058- 0.54; Total hardness, 10.01-24.0; Bicarbonate, 930- 1900; Salinity, 2418.7- 7581.0 and Sulphate, 1.48- 3.44. Temperature was 27.2 - 31.6

Figure 1. Map of Obagi flow station showing the study area

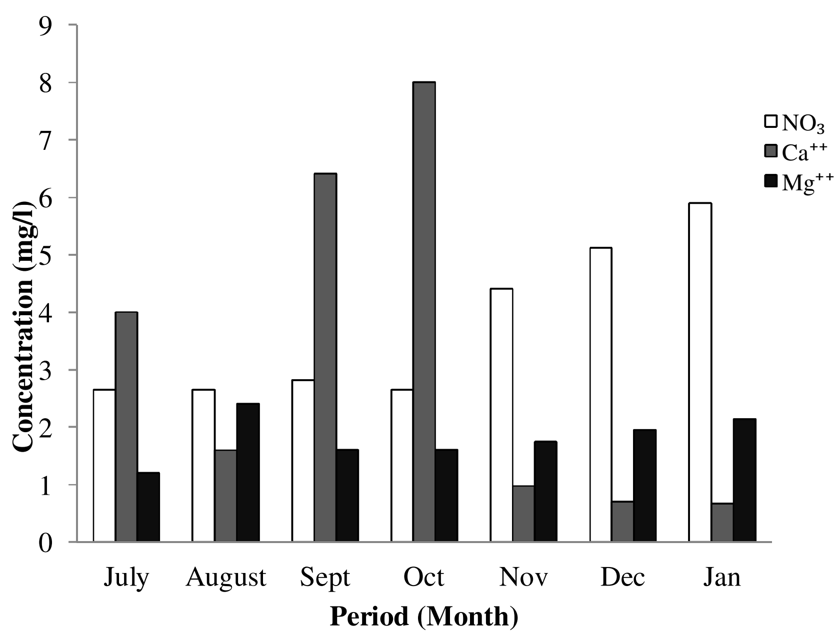

Figure 2. Seasonal profile of some ions (NO3, Ca2+ and Mg2+) of the flare pit

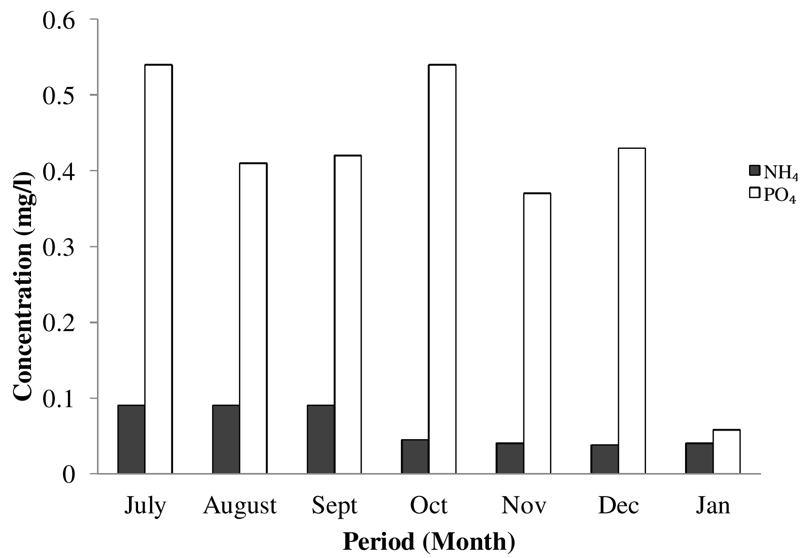

Figure 3. Seasonal profile of some ions (NH^+_4 and $PO_{4)}$ of the flare pit

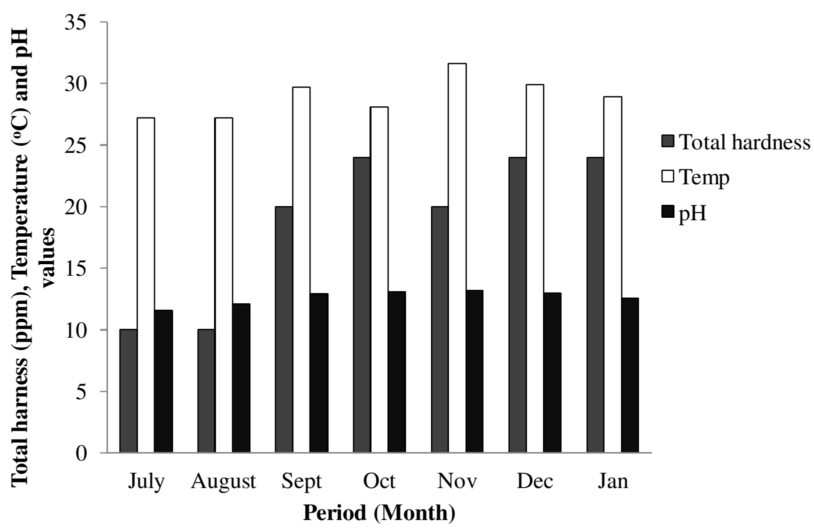

Figure 4. Total hardness,Temperature and pH profiles of the Obagi flare pit

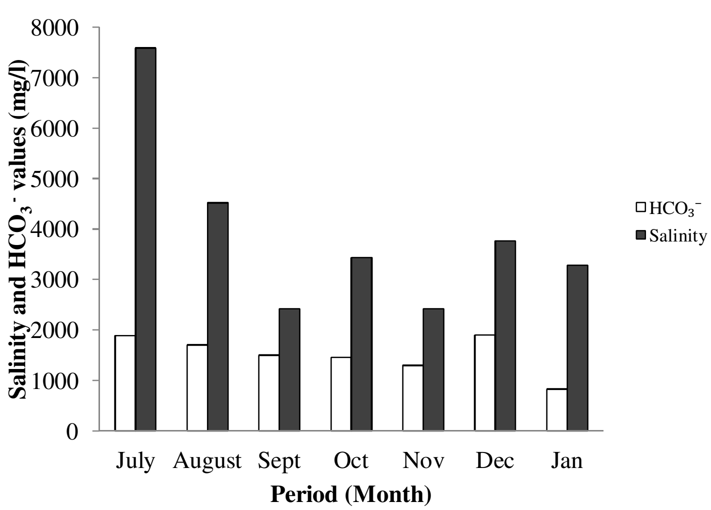

Figure 5. Salinity and HCO3- profile of the Obagi flare pit

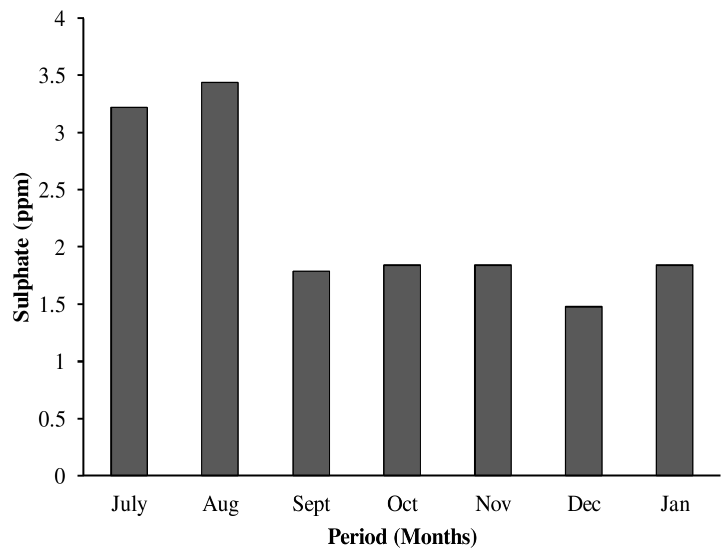

Figure 6. Sulphate concentration of the Obagi flare pit

Table 1. Concentration of heavy metals and hydrocarbons detected in the Obagi flare pit

Metals	July (ppm)	November (ppm)
Iron	0.123	0.090
Manganese	0.008	0.006
Nickel	0.022	0.167
Cadmium	0.017	0.001
Chromium	0.137	0.282
Lead	0.005	0.001
Vanadium	8.75	8.10
Total hydrocarbon	90.0	90.0

while pH was 11.6 - 13.2. Some of the results were at variance with the allowable limit of the World Health Organization (WHO). Table 1 depicts the result on the concentration of heavy metals in the flare pit. The heavy metals detected include iron, manganese, nickel, cadmium, chromium, lead and vanadium. Total hydrocarbon was 90.0mg/l. Within the period analysed total hydrocarbon remained relatively uniform while vanadium, iron, manganese, cadmium and lead had higher values in July and least in November. Only chromium and nickel gave lower values in July and higher in August. The highest in occurrence among the metals is vanadium and lead was the least in occurrence.

The Microalgae isolated from the Obagi flare pit waste water were six phyla with nineteen genera (Table 2).Only *Oscillatoria* occurred throughout the period, *Cystodinium* had six occurrences except in September, *Synechococcus* had five occurrences except in October and November and *Microcystis* had the same occurrence with *Synechococcus* except in August and October. Organisms that had four occurrence includes; *Nostoc* except in July, August and November; *Dunaliella* except in August, September and October. Those that had three occurrences were *Anabaena* in September, October and December, *Scenedesmus* in October, November and January; *Chlorogonium* in July, October and November and Heterochloris in July, August and September. *Chrysococcu*s occurred twice in July and August, along with *Euglena* in August and November and *Chlorobotrys* in October and November. But *Spirulina*, *Chlamydomonas* and *Cryptomonas* were isolated in July only, *Gleotrichia* in November, *Cosmarium* in August and *Rhizochloris* in October. There was no clear–cut pattern of occurrence of these microalgae within the season. *Oscillatoria, Cystodinium, Synechococcus* and *Microcystic* were very prominent throughout the seasons. But *Spirulina, Chlamydomonas, Cryptomonas, Cosmarium, Rhizochloris* and *Gleotrichia* occurred mainly in one season only, while *Nostoc, Dunaliella, Anabaena*, *Scenedesmus Chlorogonium* and *Heterochloris* were

Table 2. Microalgae identified in the Obagi flare pit

Microalgae	July	August	September	October	November	December	January
Cyanophyta							
Oscillatoria	+	+	+	+	+	+	+
Nostoc	-	-	+	+	-	+	+
Synechococcus	+	+	+	-	-	+	+
Anabaena	-	-	+	+	-	+	-
Spirulina	+	-	-	-	-	-	-
Gleotrichia	-	-	-	-	+	-	-
Microcystis	+	-	+	-	+	+	+
Chlorophyta							
Chlamydonomas	+	-	-	-	-	-	-
Dunaliella	+	-	-	-	+	+	+
Scenedesmus	-	-	-	+	+	-	+
Cosmarium	-	+	-	-	-	-	-
Chorogonium	+	-	-	+	+	-	-
Cryptomonas	+	-	-	-	-	-	-
Chrysophyta							
Chrysococcus	+	+	-	-	-	-	-
Euglenophyta							
Euglena	-	+	-	-	+	-	-
Phaenophyta							
Heterochloris	+	+	+	-	-	-	-
Rhizochloris	-	-	-	+	-	-	-
Chlorobotrys	-	-	-	+	+	-	-
Pyrrophyta							
Cystodinium	+	+	+	-	+	+	+

Key: + = detected; - = not detected.

found within a season at a time. *Oscillatoria* was predominant throughout the investigation while *Spirulina, Chlamydomonas, Cryptomonas, Gleotrichia, Cosmarium and Rhizochloris* were the least in occurrence (Table 3).

DISCUSSION

In any environment, variations exist within the different environmental compartments with respect to physical, chemical and biological characteristics. Ecological conditions can be seasonal and atimes help to determine the abiotic and biotic components and structural diversity of a given area (Onwugbuta-Enyi *et al.,* 2008). The values obtained from the study reveal some of the physico-chemical constituents (including heavy metals) and microalgae isolated from the flare pit which receives oil pollutants and gas flares with attendant toxic smoke and other environmental hazards. The chemical constituents of the waste water from the flare pit revealed that the waste water of Obagi flare pit is composed of traces of nitrate, ammonia, sulphate, bicarbonate, calcium, magnesium in values that are within the acceptable limit of WHO, but phosphate, salinity, calcium/total hardness values are higher than the permissible limits of WHO and might be detrimental to the health of the community and the ecosystem at large (World bank, 1995). Excess nitrate and phosphorus contribute to harmful algal blooms (eutrophication) and can lead to a biologically unproductive zone (WHO, 2004; WHO/UNICEF, 2005). The Mixing of the brackish water with inflowing freshwater might lead to only slight temperature variations with the seasons. This is consistent with the trends reported in previous studies within the Niger Delta (RPI, 1985; Erondu and Chinda, 1991). Tree shade made around the flood plain with the thick forest of trees and raffia surrounding the area, could be significant in regulating the temperature profile (Elakhame, 1995).

The pH value measured in both seasons were high and shows that the water is generally alkaline, this may be due to the waste water disposed into the pit which can support algal bloom. Other physico-chemical analysis such as salinity supports the brackish nature of the water body. This flare pit thus, encourages effective algal bloom and rich primary productivity. On the other hand

Table 3. Frequency of occurrence of genera of microalgae in the Obagi flare pit

Microalgae	Periods of occurrence	Occurrence (n=55)	%frequency of occurrence
Oscillatoria	July, August, September, October, November, December, January	7	12.73
Cystodinium	July, August, October, November, December, January	6	10.91
Synechococcus	July, August, September, December, January	5	9.09
Microcystic	July, September, November, December, January	5	9.09
Nostoc	September, October, December, January	4	7.27
Dunaliella	July, November, December, January	4	7.27
Anabaena	September, October, December	3	5.45
Scenedesmus	October, November, January	3	5.45
Chlorogonium	July, October, November	3	5.45
Heterochloris	July ,August, September	3	5.45
Chrysococcus	July, August	2	3.64
Euglena	August, November	2	3.64
Chlorobotrys	October, November	2	3.64
Spirulina	July	1	1.82
Chlamydomonas	July	1	1.82
Cryptomonas	July	1	1.82
Cosmarium	August	1	1.82
Rhizochloris	October	1	1.82
Gleotrichia	November	1	1.82

the algal blooms could lead to low dissolved oxygen, presence of cyanotoxins, tastes and odour - causing compounds, leading to disruption in settling in terms of coagulant demand and sludge fouling (Baudin *et al*., 2006). Oil production contributes to air pollution in the form of flaring, the burning of natural gas extracted along with crude oil. Flaring as way to dispose of the natural gas may end up releasing potent greenhouse gases such as water vapor, carbon dioxide, methane, nitrous oxide, ozone and chlorofluorocarbons (CFCs) which fill the air with smoke and covers the land in soot, contributing to the rising acidity of the rain (Moffat and Linden, 1995). Iron, nickel, cadmium and chromium are high within the periods analysed and are above the permissible limit of WHO. Many of these heavy metals are toxic to humans and aquatic life (WHO, 2004; WHO/UNICEF, 2005). High levels of nickel, cadmium and chromium could lead to toxicity to kidney, cancer, poor development in infants, breakdown of the central and peripheral nervous system and interference with vitamin metabolism (IPAN, 2009).

The phytoplankton investigations revealed low phytoplankton density which is a feature of tropical black water especially the black water types of the Niger Delta regions which are known to be less productive than other black water types. The study revealed about 19 genera of phytoplankton. Onuoha (1980) reported about 29 genera of phytoplankton in the Bonny River, Nwadiaro and Ezefili (1986) reported low density of algal flora in the New Calabar River. Our observations are in line with results conducted on some water bodies within the Niger Delta (Hess *et al.,* 1985; RPI, 1985; IPS, 1986; Powell and Chinda, 1986, Abu and Epegu, 2006).The frequency of occurrence of the microalgae showed that *Oscillatoria* (12.73%) was predominant throughout the period, while *Spirulina, Chlamydomonas, Cryptomonas, Cosmarium, Rhizochloris* and *Gleotrichia* were the least isolated at 1.82% of occurrence. Different seasons encourage the growth and proliferation of certain microalgae. The response of these microalgae to seasonal changes may be due to their nutritional and physiological potentials which could be altered by the different seasons and affect their survival. Changes in environmental chemistry and biodiversity can give some organisms a window of opportunity to flourish. Most of these microalgae (*Spirulina, Dunaliella*, and *Scenedesmus)* are used as food supplement because of their excellent nutrient composition and digestibility, and are commercially available in powder, flakes or granule forms and as tablets and capsules *(*Brown*, et al.,* 1997*;* Spolaore*, et al.,* 2006; Barrow and Shahidi*,* 2008). Some strains of *Nostoc* and *Anabaena* are consumed as human food in some parts of the world. With high fibre and moderate protein these microalgae have potential use as a new dietary fibre source. Thus, they can play an important physiological and nutritional role in human diet, as feed for aquatic animals and as biofertilizers because their high concentrations of potassium and trace elements improve crop production. *Nostoc* and *Anabaena* have been used in the large scale production of enzymes; they are rich sources of polysaccharides and lipids with varied properties. An immunopotentiating compound with male anti-fertility without being toxic to other systems was found in the extracts of *Oscillatoria* with other immune-modulatory activities. *Oscillatoria* are used in waste treatment since the algae bring about oxygenation and mineralization. *Chlamydomonas* atimes switch from the production of oxygen to the production of hydrogen which is becoming a source of biofuel (Meeks *et al*., 2005). Microalgal pigments including chlorophylls and phycocyanins of the cyanobacteria are becoming veritable tools for remote sensing of biomass (Simis *et al.,* 2007; Bryant, 1981). Biomass remote sensing is useful in water quality management.

ACKNOWLEDGEMENT

The authors thank the plant manager, Obagi flow station, Mr. Aikodje, all the laboratory staff at the Obagi flow station, Ogbogu, Mr. Hycinth Nworgu of the General Microbiology laboratory of University of Port Harcourt, for technical assistance, Mr. Japheth onwuegbu of the department of Plant Science and Biotechnology, University of Port Harcourt, for physico-chemical analysis.

REFERENCES

Abu GO, Epegu CD (2006). Isolation of Microalgae from fresh water ponds at the African Regional Aquaculture centre Aluu and the laboratory assessment of their economic potentials. Niger. J. Microbiol. 20:817-823.

American Public Health Association (APHA) AWWA and WPCF (1998). Standard methods for the Examination of Water and Waste Water, 20th edition. Washington DC, USA.

Apt KE, Behrens PW (1999). Commercial Development in Microalgal Biotechnology. J. Phycol. 35: 215-226.

Barrow C,Shahidi F (2008). Marine neutraceuticals and Functional foods. CRC Press, Taylor and Francis Group.

Baudin I Cagnard O Grandguillaume JJ, Do- Quang Z (2006). Algae and associated toxins and metabolites: methodology for risk assessment and risk management. Water Practice and Technol. 1: 4.

Brown MR Jeffrey SW Volksman JC, Dunstan GI (1997). Nutritional properties of Microalgae for marine culture. Aquaculture 151(1-4): 315-331.

Bryant DA (1981). The photo regulated expression of multiple phycocyanin species. Eur. J. Biochem. 119:425-429.

Burris JE (1977). Photosynthesis, Photorespiration and Dark respiration in eight species of algae. *Marine Biol*. 39: 371-379.

Chisti Y Grima EM Belarbia EH Fernandez FGA, Medina AR (2003). Recovery of Microalgal Biomass and Metabolites: Process Options and Economics. Biotechnol. Adv. 20: 491-515.

Cohen Z (2000). Chemicals from microalgae. Taylor and Francis Publishers. Pp. 419.

Department of Petroleum Resources (DPR) (1991). Environmental Guideline and Standards for the Petroleum Industry in Nigeria. Ministry of Petroleum Resources, Lagos. Pp. 30 - 37.

Elakhame LA (1995). The biology and ecology of fishes in Epie Creek floodplain with particular reference to Odediginni Lake, Niger Delta Nigeria. PhD Thesis University of Port Harcourt.

Erondu ES, Chindah AC (1991). Variations in the physico-chemical features and phytoplankton of the New- Calabar River, at Aluu, Rivers State, Nigeria. Nigerian Institute for Oceanography and Marine Research (NIOMR). Technical Paper No 75.

Guillonneau N Fontaine G Gory JY Iwuoha,S Ahmed H, Ekpenyong D (2009). Obagi-Present and Future Challenges of a mature oil field. Nigeria Annual International Conference and Exhibition, Abuja, Nigeria.

Hess S Dunchan P, Powell CB (1985). Plankton of the Niger Delta. Paper presented at the Nigerian National Petroleum Company Conference, in Kaduna. pp. 1-15.

Institute of Public Health Analyst of Nigeria (IPAN) (2009). Nigerian standards for drinking water quality. In: IPAN News 10(34): 19–21.

Institute of Pollution Studies (IPS) (1986). Oshika oil spill (NAOC Pipeline). Environmental Impact Assessment Report No. RSUST/IPS/R/86/102:1-178.

Jones BE, Grant WD, Duckworth AW, Owenson GG (1998). Microbial diversity of Soda Lakes. Extremophiles 2: 191–200.

Lee CG (1999). Calculation of light penetration depth in photobioreactors. Biotechnol. and Bioprocess Engineering 4: 78–81.

Marshall TF, Devinny JS (1988). The Microbial ecosystem in petroleum wasteland treatment. Wat. Sci. Tech. 20(11/12): 285–291.

Meeks JC (2005). Cyanobacterial biodiversity and potential applications in biotechnology. Curr. Sci. 89(1): 52-57.

Moffat D, Linden O (1995). Perceptions and Reality: assessing priority for sustainable Development in the Niger River Delta. Ambio 24(7-8): 527.

Molina E, Contrevasa A, Garcia F, Merchok JC (1999). Influence of sparger on energy dissipation, shear rate and mass transfer to sea water in a concentric tube airlift bioreactor. Energy and Microbiol. Technol. 25: 820–830.

Nwadiaro CS, Ezefili (1986). A preliminary check list of the phytoplankton of New Calabar River, lower Niger Delta. Hydrobiological Bulletin. 19(2): 133-138.

Obire O (1988). Studies on the biodegradation potential of some microorganisms isolated from water systems of two petroleum producing areas in Nigeria. Nig. J. Bot. 1: 81-90.

Obire O (1988). The Suitability of various Nigerian petroleum fractions as substrate for bacterial growth. Discovery and Innovations 5: 45-49.

Obire O, Wemedo SA (2002). Seasonal effects on the bacterial and fungal population of an oilfield wastewater-polluted soil in Nigeria. J. App. Sci. and Env. Mgt. 6(2): 17-21.

Olaizola M (2003). Commercial development of microalgal biotechnology from the test-tube to the market place. Biomolecular Engineering, 1-8.

Onuoha GC (1980). Phytoplankton and oil pollution in Bonny River, Nigeria, M.Sc. Thesis. University of Port Harcourt, Nigeria. pp.79.

Onwugbuta-Enyi J, Zabbey N, Erondu ES (2008). Water quality of Bodo Creek in the Niger Delta Basin. Adv. in Environ. Biol. 2(3):132-136.

Powell CB, Chinda AC (1986). Variations in phytoplankton communities and densities along the middle and upper reaches of the Bonny/New Calabar estuary, Paper presented at the Man and Biosphere Workshop on Wetlands of Port Harcourt. pp. 20.

Romano I, Bellitti MR, Nicholaus B, Lama L, Manca CM, Pagnotta E, Ganbacorta A (2000). Lipid profile a useful chemotaxonomic marker for classification of a new cyanobacterium in *Spirulina* genus. Phytochem. 54: 289-294

RPI (1985). Research planning institute, inc. Columbia, USA. Environmental baseline studies for the establishment of control criteria and standards against petroleum related pollution in Nigeria. Report No. RPI/R/84/4/15-7.

Simis GHS, Ruiz-Verdu A, Dominguez-Gomez JA, Pena-Martinez R, Peters SWM, Gons HJ (2007). Influence of phytoplankton pigment composition on remote sensing of cyanobacterial biomass. Remote Sensing of Environ. 106: 414-427.

Tandeau de Marsac N, Houmard J (1993). Adaptation of cyanobacteria to environmental stimuli: new steps towards molecular mechanisms. FEMS Microbiol. Rev. 104:119-190.

Tramper J, Battershill C, Brandenburg W, Burgess G, Hill R, Luiten E, Muller W, Osinga R, Rorrer G, Tredici M, Uriz M, Wright P, Wijffels R (2003). What to do in marine biotechnology. Biomolecular Engineering, 1-5.

World Bank (1995). Nigeria Strategic options for Redressing Industrial pollution. World Bank, Industry and Energy Division. West Central

Africa Dept. Vol. 2, Annexes, 60- 62.
World Health organization (2004). Guidelines for Drinking Water Quality. 3rd Edth 47. Vol. 1. Recommendation. WHO: Genera, Switzerland. pp. 515.
WHO/UNICEF (2005). Water for life. Making it happen. A Decade for Action 2005-2015. Genera, 44. at. http://www.Who.Int/water sanitation health/water for life. pdf