Physicochemical Properties and Algal Dynamics in Culture: A Case Study of Toluene Pollution

* E. M. Denise, E. N Akpan, M. C. Anyadike, and E. L. Ezendiokwelu

Department of Botany and Ecological Studies, University of Uyo, Uyo, Akwa Ibom State, Nigeria.
*Corresponding Author: mukoroemmanuel01@gmail.com and mariac591@yahoo.com; 08067043869 and 08160260285

ABSTRACT
This work evaluates the physicochemical properties and algal dynamics in culture. A batch culture of algae constituted with 20% toluene and 0.250g of poultry dropping was subjected to natural inoculation by algae for a period of 6 weeks, physiochemical parameters were analyzed using the methods described in APHA. Samples were analyzed every other day for six weeks. Volume ranged between 1000ml and 6327ml. Temperature of the culture ranged between 29°C and 34°C while pH values range from 7.2 to 9.6. There was no significant effect of toluene on the physicochemical parameters (volume, Temperature and pH) and biological oxygen demand (BOD) content of the culture (P > 0.05). The colonization of algae in the culture (toluene Conc. = 20%) becomes only evident at the 20th day of the study. The algal divisions present in the culture include the Cyanophyta, Euglenophyta, Chlorophyta and Desmids. Cyanophyta were represented by Oscillatoria, Lyngbya and Chroococcus, Euglenophyta was represented by Phacus, Chlorophyta were represented by Chlorella, Closterium and Hydrodicton, while Desmids was represented by Desmidium aptogonium. The Cyanobacteria and Euglenophytes were the most dominant algal species. The result implies that toluene did not affect the physicochemical properties of algal culture but the colonization of different algal forms. The algal dynamics structure could suggest bioremediation of toluene. This result could be useful in studying algal dynamics in aquatic ecosystem.

Key words: Physiochemical properties, Algal dynamic, Bioremediation, Colonization, Dominant, Ecosystem

INTRODUCTION
The aromatic compounds found in petroleum are an important group of abstracted pollutants. These aromatic compounds are introduced into the environment from various sources such as natural oil seeps, refinery waste products and emissions, oil storage wastes, accidental spills from oil tankers, petrochemical industrial effluents and emissions, and coal tar processing wastes, etc. Petroleum hydrocarbons can rapidly migrate from the site of contamination and adversely affect terrestrial and aquatic ecosystems and humans (Fishbein, 1985).

Phycoremediation is a novel technique that uses algae to clean up polluted water and soil. It takes advantage of the alga's natural ability to take up, accumulate and degrade the constituents that are present in their growth environment. The oxidation ponds is a cheaper alternative treatment process which is quite efficient in removing the pollutants where meeting the oxygen requirement is done by algal photosynthesis, the use of algae as a waste treatment, was pioneered by Oswald et al., (1953) and a good deal of work on the treatment of waste water by algal species has also been done by Olugin (2003) and Zhang et al., (2008). In recent years, the use of microalgae in bioremediation of colored waste water has attracted great interest due to their central role in carbon dioxide fixation (Huang et al., 2010). Although, bacteria play a key role in the biodegradation of organic pollutants, recent studies have indicated that in
addition to providing oxygen for aerobacterial biodegraders, microalgae can also degrade organic pollutants directly (Mallick, 2002). There is usually a positive correlation between the seasonal changes in physicochemical and biochemical characteristics of water and the productivity of a lentic water body. The distribution and abundance of microalgae in lentic water are controlled by a wide range of physical, chemical and biological factors such as Temperature, Volume, pH, Dissolved oxygen (DO) and Biochemical oxygen demand (BOD) (Mathias et al., 2011). The response of algae to changing properties of water due to loading of substances from inland water flow has been reported by (Wan, 2010). But the purpose of this work is to access the effect of toluene on the physicochemical and algal dynamics.

**Source of Pollution**
Toluene is primarily manufactured by catalytic reforming of petroleum. It is a common gasoline additive and is used in the production of various organic compounds. Recently, they have been reported as component of contaminants present in surface and ground waters, which usually originate from the leakage of underground petroleum storage tanks, direct discharges of industrial effluents especially from chemical production and refinery sites, spills at oil production, pipelines and distribution terminals, industrial wastewaters and atmospheric deposition. White et al., (2009) opined that toluene may originate from biogenic sources in coastal waters. More so, large volumes of petroleum products such as gasoline, engine oil, naphthalene, benzene, toluene and other industrial effluents are either directly or indirectly discharged into the aquatic environment thereby causing ecological imbalance in the ecosystem (Kori-Siakpere, 2000).

Volatile is an important predominant removal process for toluene and other abstractly hydrocarbons present in the aqueous environment (Wakeham, 1983). Fishbein, (1985) estimated that more than 6 million tons of toluene entered the environment annually. Priyanka et al., (2013) in their work on the physicochemical characteristics of a freshwater pond of Orai, reported that the pH value of the pond showed an alkaline trend with a few variations.

Noel and Rajan, (2014) in their studies on the use of Cyanobacteria as a source of phycoremediation from textile industry effluent reported that the pH of the effluent sample treated with *Anabaena variabilis* showed a drastic change from 7.3 to 9.6. Increase in pH has also been observed in the effluent sample treated with other Cyanobacterial species. Kotteswari et al., (2007) reported a pH change from 5.62 to 9.82. Sanjay and Goswami, (2014), in their work on photocatalytic oxidation of toluene in water from an algae pond with high dissolved oxygen content, reported that dilution which increases the volume of the water is often necessary in the photocatalytically treatment of pollutants. An advantage of dilution is that the dissolved oxygen content of the effluent is raised; on the other hand dilution increases the volume to be these. It has been pointed out that the amount of rainfall in the water bodies play a significant role in regulating the various seasonal biological rhythms and thus, raise the level of aquatic systems with the concentration of certain chemical substances which in turn influence the fluctuations in the quantity and quality of phytoplankton (Hulyal and Kaliwal, 2009). The temporal variations of phytoplankton in tropical systems are related to differences in rainfall (Subhabrata, 2012; Chellappa et al., 2008; Dantas et al., 2008).

Brijesh et al., (2012), in their research on the biodegradation of toluene under seasonal and diurnal fluctuations of soil-water temperature, reported that toluene degradation rate is increased with increase in temperature. At low temperature, usually there is reduced volatilization and decreased water solubility of Benzene, Toluene, Ethylbenzene and Xylene (BTEX), and thus delayed onset of biodegradation process (Margesin and Schinner, 2001). On the other hand, solubility and thus bioavailability of BTEX compounds are enhanced at elevated temperatures.

Rai and Muniyandi, (1981) reported in their one year phycological study of a high altitude pond with a view to the study of algal dynamics as affected by physicochemical characteristics of the water. This has revealed the presence of 34 genera of algae belonging to Chlorophyta, Bacillariophyta, Cyanophyta, and Eunlenophyta. Chlorophytes were the most dominant and diatoms the most frequent representatives of algae.
Senngar et al., (2011) in their research on the application of phycoremediation technology in the treatment of sewage water to reduce pollution load reported that the oxidation of the pond is brought about by the complex symbiosis of algae and bacteria. On the basis of percentage abundance the algal species were classified into three categories that is highly prominent (abundance more than 10%), prominent (between 3-10%) and less prominent (less than 3%), thus during their experiment, Oscillatoria spp and Phormidium corium multiplied and achieved their position in most prominent category while Gloeocapsa gelatinosa and Synedra affinis became less prominent.

MATERIALS AND METHOD

Hydrocarbon

Toluene (C₇H₈) also called methylbenzene, toluol and phenylmethane is a volatile organic compound (VOC). At room temperature, it is a colourless, sweet smelling water insoluble liquid that has a smell associated with paint thinners with melting point -95°C and boiling point of 111°C. Its vapour pressure is 3.78kPa at 25 °C, relative density is 0.8623 g/cm³ at 15.6 °C and its solubility in water is 535 mg/litre (WHO, 2004; McKeown, 2015). It belongs to a class of chemicals known as the monoaromatic hydrocarbons, like ethylbenzene and xylene, ‘toluene is alkyl benzene’ by having one methyl group (CH₃) added on the benzene ring (substitute for hydrogen).

Hydrocarbon source

The hydrocarbons used for this work was Toluene. It was obtains from Thomas Gold Ventures in Benin, Edo State, Nigeria

Preparation of 20% Toluene

20ml of Toluene was added to 80ml of distill water which gives a concentration of 20% toluene; this was added to 900ml of distill water obtain a 1000ml of treatment culture

Experimental Set Up

The experiment was set up with yellow custard plastic buckets in replicate of three which comprises of control (without any addition of toluene) and the treatment culture with 20% toluene. These were kept in the field for natural inoculation of algal spore

Growth Substrate

The growth substrate used was poultry droppings which were added in all the experiment both in the control and the treatment samples to stimulate and enhance the growth of algae in the samples.

Preparation of Growth Nutrient / Medium

Growth medium was prepared in accordance with the method applied by Kadiri and Emmanuel, (2003). 0.250g of poultry droppings was dissolved in 1000ml of distill water and left over night, 40ml of the solution was then added to both the control and the treatment samples as nitrogen source.

Physiochemical Analysis

Temperature: This was done with the use of mercury thermometer normally carried out in the morning. The thermometer was dipped into the water sample and completely immersed in water for 5mins.

pH: This was done with the use of pH meter. The pH meter was dipped into the water samples and the values were record when stable.

Volume: This was measured for every 10days to note the rate of dilution in the treatment sample and the amount of water added by rainfall.

Microscopic Identification of Algae species

A box sample was made from the three replicate for microscopic examination from 5ml of samples collected from each of the three replicates to obtain a box 15ml sample of the water sample from control and treatment culture separately.

Preservation of Sample

Immediately after collection, 5 drops of Lugol’s iodine and 50% formaldehyde was added to fixed the algal cells.
Sample Sedimentation.
5ml of the sample was centrifuged using Model 80-2 centrifuge. A small quantity of the sediment bottom of the centrifuge tube was obtained for the microscope study, photo microgram or picture of the different algal cells which were observed.

Cell count: Population density of the various algal forms in the various divisions was estimated using the haemocytometer. Aliquot containing uniformly mixed microalgae were put into the haemocytometer groove and a cover slip placed on it to ensure that all grooves contained enough sample without air bubbles. A Leitzorthoplan compound microscope was used for counting. Three mounts were counted and the average number of cells per ml estimation using the formula below.

\[ \text{Number of cells per ml} = \frac{X \times 10^7}{125} \]

Where: \( X \) = Average number of cells in sixteen big squares of the haemocytometer.

RESULTS
The results of the effect of toluene on the physicochemical properties of a culture are shown in Table 1, 2 & 3. The results show that there was relatively no significant difference in temperature between the control experiment (28°C - 34°C) and the treatment culture (29°C - 35°C). The pH values increase with increased in the colonization of algae in both the control and treatment culture. The volume of the samples persistently increased throughout the investigation period in both samples.

### Table 1: pH Values of the culture during the study period

<table>
<thead>
<tr>
<th>Days of culture</th>
<th>Control (0%)</th>
<th>Toluene (20%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st day</td>
<td>7.53</td>
<td>7.2</td>
</tr>
<tr>
<td>10th day</td>
<td>7.30</td>
<td>6.96</td>
</tr>
<tr>
<td>20th day</td>
<td>8.13</td>
<td>7.6</td>
</tr>
<tr>
<td>30th day</td>
<td>9.37</td>
<td>9.3</td>
</tr>
<tr>
<td>40th day</td>
<td>9.5</td>
<td>9.6</td>
</tr>
</tbody>
</table>

Note: 0% is the Control (without any hydrocarbon)
20% is the Treatment culture (with 20% toluene)

### Table 2: values of volume of the culture for the period of study

<table>
<thead>
<tr>
<th>Days of culture</th>
<th>Control (0%)</th>
<th>Toluene (20%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st day</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>10th day</td>
<td>1196</td>
<td>1332</td>
</tr>
<tr>
<td>20th day</td>
<td>3374</td>
<td>4030</td>
</tr>
<tr>
<td>30th day</td>
<td>4881</td>
<td>4748</td>
</tr>
<tr>
<td>40th day</td>
<td>6040</td>
<td>6327</td>
</tr>
</tbody>
</table>

Note: 0% is the Control (without any hydrocarbon)
20% is the Treatment culture (with 20% toluene)

### Table 3: Temperature values of the culture for the period of study

<table>
<thead>
<tr>
<th>Days of culture</th>
<th>Control (0%)</th>
<th>Toluene (20%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st day</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>10th day</td>
<td>34</td>
<td>35</td>
</tr>
<tr>
<td>20th day</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>30th day</td>
<td>28</td>
<td>32</td>
</tr>
<tr>
<td>40th day</td>
<td>34</td>
<td>33</td>
</tr>
</tbody>
</table>

Note: 0% is the Control (without any hydrocarbon)
20% is the Treatment culture (with 20% toluene)
Algal Composition
In the treatment culture, eight genera represented the algal species in the division: Cyanophyta, Chlorophyta, Euglenophyta and the Desmids were observed to be present. The Cyanophyta were represented by Oscillatoria spp, Lyngbya spp and Chroococcus sp, the Chlorophyta were represented by Chlorella spp, Hydrodicton spp and Closterium spp, the Euglenophyta was represented by Phacus spp, And the Desmids was represented by Desmidium aptogonium. Also in the control experiment, the algal composition were represented by the algal species in the division; Chlorophyta, Euglenophyta and Cyanophyta. The Chlorophyta were represented by Chlorella spp and Closterium spp, the Cyanophyta was represented by Oscillatoria spp, And the Euglenophyta was represented by Phacus spp.

There was no significant effect (P > 0.05) in the Temperature, Volume and pH content of both samples. Hence, toluene did not affect the physicochemical properties of a culture in this study.

DISCUSSION
Algae response to changes in the physical and chemical properties of water due to loading of substances from inland water flow (Wan, 2010). In this study, the persistent increase in the volume of the sample could be due to the addition of water through precipitation in both the control experiment and treatment culture. Also there was slight increase in the volume of the treatment culture than observed in control experiment which could be probably due to the effect of evaporation and the rate of dilution. The pH values varied tremendously throughout the investigation. This variation could be due to inorganic factor. This was corroborated with the growth of algae as observed green colouration of the culture by various species of algae. The colouration could have affected the inorganic content of the culture media due to utilization of inorganic iron for photosynthesis. The colouration becomes dense with increase in the volume of the culture and maximum colonization of the pond with algal species. The variation observed in temperature values of the samples could probably be due to the effect of time differences in collecting samples for readings. The treatment culture showed gradual increase in temperature which was not the case in the control experiment. This increase could be due to increase in degradation or breakdown of toluene; this is in accordance with the report by Brijesh et al., 2012 who reported the degradation rate of toluene in water. He opined an increased in temperature. The temperature range of the treatment culture throughout the investigation period was relatively similar to that of the control experiment this finding could be due to the volatile nature of the hydrocarbon (toluene).

CONCLUSION
It is extremely rare that a single microorganism is capable of completely degrading a pollutant or a mixture of xenobiotics. Under environmental conditions, the combined action of microalgae and other microorganisms might be a rather important process for the elimination of these undesired compounds from the environment. The degradation of pollutants under these conditions usually involves the combined actions of two or more microorganisms. From this current study, is obvious the combined actions of different algal species from different divisions were able to colonize the toluene polluted environment which is a sign that these species may be capable of degrading or bioremediating toluene from the environment. We therefore recommended the investigation of these species in bioremediation protocol.

REFERENCES


