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Discovery

Preliminary investigation of algal forms and colonization pattern of algae in an environment polluted with Toluene

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ABSTRACT

Release of low molecular aromatic hydrocarbon into natural waters brings severe consequences to our environment. Unfortunately very limited information is available regarding the limnological status of water contamination with these pollutants. This work evaluated the effect of toluene on algal forms and their colonization pattern in culture. In the study, the colonization of algae in the culture (toluene Conc. = 20%) started on the 20th day of the study. The algal divisions present in the culture in a 40 day investigation 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. In the order of colonization, the Cyanobacteria were the pioneer species followed by the Euglenophytes and the Chlorophytes. The Desmids were last to colonized the culture during the period under study. The species colonization reflects the bioremediation potential of hydrocarbon of the various algal forms. Microscopic quantification of algal density using haemocytometer reveals the algal species in the division Cyanophyta were the most do in ant species in the treatment culture (57.2%). While in the control experiment, the algal species of the division Euglenophyta and Chlorophyta were the most dominant species at the early age of the culture. These were represented by (54.5%) and (36.4%) respectively. However, at the mid and terminal age of the culture, the algal species of the divisions Cyanophyta and Euglenophyta were the most dominant, these were represented by (57.2%) and (21.4%) respectively. In the control experiment, the algal species in the division Euglenophyta maintained their dominant nature with a 54.5% population. Result of this study could be useful in understanding colonization pattern and as such could be a vital tool in management of toluene polluted environment and general clean up of the environment with respect to toluene and other related crude oil hydrocarbons in bioremediation protocol.

Key words: Hydrocarbon, Limnological status, Environmental cleanup, Bioremediation, Colonization pattern

1. INTRODUCTION

Algae occur ubiquitously in abundance throughout aquatic ecosystems. They play a key role in primary production in aquatic habitat. Algae are morphologically simple, chlorophyll-containing autotrophic organisms that range from microscopic and unicellular (single-celled) to very large and multicellular. The algal body is relatively undifferentiated and there are no true roots or leaves (Guiry, 2012). 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 has been reported by (Wan, 2010).

Toluene (C_7H_8) also called methylbenzene; toluol and phenylmethane is a volatile organic compound (VOC). At room temperature, it is colorless, sweet smelling water that has a smell associated with paint thinners with melting point -95^oC and boiling point of 111^oC. Its vapour pressure is 3.78kPa at 25 °C, relative density is 0.8623 g/cm^3 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 (CH_3) added on the benzene ring (substitute for hydrogen).

Hydrocarbon contamination in the environment is a serious problem whether it comes from petroleum, pesticides or other toxic organic matter. Environmental pollution caused by petroleum is of great concern because petroleum hydrocarbons are toxic to all forms of life (WHO, 2004). Pollution of water due to the release of hydrocarbon is a major public health concern and therefore remediation of these natural resources is needed to eliminate risk pose to the environment by the mono aromatic hydrocarbon such as benzene, toluene, ethylbenzene and mixture of xylene are of particular concern because of their high water solubility which enable them to spread in the surface. Water bodies are currently being degraded by both natural and anthropogenic activities, which deteriorate its quality, altered its functions and affect the ecological balance. The release of low-molecular aromatic hydrocarbons into natural waters brings severe consequences to our environment. Unfortunately very limited information is available regarding the effect of these pollutants on water quality. The aim of this work is to determine the colonizing pattern of freshwater algae in water polluted with toluene.

2. LITERATURE REVIEW

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) opine 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).

Denise *et al*., (2017) investigated the effect of water saturated fractions (WSF) of mixtures of benzene and toluene on the growth of *Pediastrum duplex* and reported no adverse effect of WSF on the growth and biomass production at the investigated concentrations. The investigation reveal that growth and biomass production in *P. duplex* were enhance in all treatments.

Praepilas and Pakawadee (2001) investigated the potential of microalgae (*Scenedesmus quandricauda* and *Chlorella sp*.) in utilizing industrial waste water as a cheap nutrient for their growth. The culture gave the highest lipid content at 18.58% and 42.86% in cases of *S. quandricauda* and *S. obliquss* in addition, under salt stress (1.0M NaCl) *S. obliquss* demonstrate the highest lipid content at 50% which was more than the case of no NaCl adding.

Stepaniyan (2008) considered the effect of crude oil on basic functional characteristics (growing speed, photosynthesis and death) of microalgae of the Barent sea using specific species (*Laminaria saccharina*, *Fucus vesticulosus*, *Ascophyllum nodosum*, *Porphyra umbilicalis*, *Palmaria palmaria*, and *Enteromorpha prolifera*) and concluded that Kelp are more resistant to the influence of oil while the rhodophytes and green algae are less resistant to oil under short term. Influence of oil toxin depressed photosynthesis and increases respiration while in a long term; the rate of growth was reportedly reduced.

Dunstan *et al*., (1975) in their work on the Stimulation and Inhibition of phytoplankton growth by low molecular weight hydrocarbons observed that in the experiment on four phylogenetically different phytoplankton exposed in culture to a range of concentrations of benzene, toluene and xylene showed a variety of growth responses for marine microalgae, and that the degree to which aromatic hydrocarbon influence phytoplankton growth varies with concentration. At low concentration of 10^4µg/l, *Amphidinium carterae* growth is significantly inhibited by benzene and toluene while xylene has no influence, *Skeletonema costatum* is not affected by benzene and toluene while xylene inhibits its growth and the green flagellated *Dunaliella tertiolecta* together with *Cricosphaera carterae* showed significant growth enhancement at lower concentrations of all the three hydrocarbons. They also reported that the concentration of 10^4µg/l appears to be a toxicity or inhibition threshold for all the three hydrocarbons in all organisms except the green algae, which are capable of good growth in solutions at the upper limit. Takáčová *et al*., (2015) on their research on the degradation of BTEX by microalgae; *Parachlorella kessleri*, monitored the effect of BTEX on the growth of *P. kessleri* and observed that after 48hours the growth of *P. kessleri* was minimally inhibited

3. MATERIALS AND METHOD

Hydrocarbon and Source: The hydrocarbons used for this work was Toluene. It was obtained 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 ofcontrol (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.

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 Leitz orthoplan compound microscope was used for counting. Three mounts were counted and the average number of cells per ml estimation using the formula below.

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

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

4. RESULTS

Changes in algal culture

On the 20th day of investigation there was a visible change in the colour of the control experiment while there was no visible colour change in the treatment culture until the 30th day. The colour change was most pronounced in the control experiment compared to the treatment cultures (fig.1 & 2).

Figure 1 An Aerial View of Experimental Setup

Fig 1.0 Changes in the control experiment as observed in the 30th day of the investigation period.

Figure 2 Changes in the treatment culture as observed in the 30th day of the investigation period

Algal study

Microscopic examination of Algal species was done using Binoculars Compound Microscope (X40) reveals species of algae at 10th, 20th, 30th and 40th day of the investigation in both control experiment and treatment culture (Table 1).

Table 1 A Forty day Comparative Study of Colonization Pattern of Algae in both Cultures

Days	Control 0 %		Toluene 20 %	
	Algal Species	**Division**	**Algal Species**	**Division**
1st	**No species**	**Nil**	**No species**	**Nil**
10th	*Chlorella spp*	Chlorophyta	Nil	Nil
20th	*Chlorella*, *Phacus,* *Closterium gracile*, *Oscillatoria*	Chlorophyta Euglenophyta Chlorophyta Cyanophyta	*Oscillatoria,* *Lyngbya* *Phacus* *Chlorella*	Cyanophyta Cyanophyta Euglenophyta Chlorophyta
30th	*Closterium,* *Phacus,* *Chlorella*	Chlorophyta Euglenophyta Chlorophyta	*Oscillatoria* *Closterium spp,* *Phacus*, *Lyngbya* *Desmidium aptogonium*	Cyanophyta Chlorophyta Euglenophyta Cyanophyta Desmids
40th	*Triploceras gracile,* *Chlorella,* *Phacus*	Desmids Chlorophyta Euglenophyta	*Chlorella,* *Hydrodicton* *Chroococcus*	Chlorophyta Chlorophyta Cyanophyta

Algal composition: In the treatment culture, eight genera represented by the algal species in the division: Cyanophyta, Chlorophyta, Euglenophyta and Desmids were observed. Cyanophyta were represented by *Oscillatoria spp, Lyngbya spp* and *Chroococcus spp*. Chlorophyta were represented by *Chlorella spp*, *Hydrodicton spp* and *Closterium spp.*Euglenophyta was represented by *Phacus* spp while the Desmids was represented by *Desmidium aptogonium.*In the control experiment, the algal species present include members of the division; Chlorophyta, Euglenophyta and Cyanophyta. The Chlorophyta were represented by *Chlorella spp* and *Closterium spp*. Cyanophyta was represented by *Oscillatoria spp* while the Euglenophyta was represented by *Phacus spp*.

Algal colonization pattern: In the treatment culture, the order of colonization is as follows, Cyanophyta were the pioneer, these were followed by the Euglenophyta. Euglenophyta were then followed by the divisions Chlorophyta and Desmids which were the last colonizer within the duration under investigation. In the control experiment, the order of colonization is as follows, Chlorophyta and Euglenophya divisions were the pioneer algal species, and these were followed by the division Cyanophyta. Microscopic quantification of algal density using the haemocytometer reveals the algal species in the division Cyanophyta were the most dominant species in the treatment culture (57.2%). While in the control experiment, the algal species of the division Euglenophyta and Chlorophyta were the most dominant species at the early age of the culture. These were represented by (54.5%) and (36.4%) respectively. However, at the mid and terminal age of the culture, the algal species of the divisions Cyanophyta and Euglenophyta were the most dominant, these were represented by (57.2%) and (21.4%) respectively. In the control experiment, the algal species in the division Euglenophyta maintained their dominant nature with a 54.5% population.

5. DISCUSSION

Changes in algal culture: The green colouration observed between days 10th and 40th in the control experiment is an evidence of growth of microscopic forms which could be due to high algae reproduction. Algal growth and species colonization is function of reproduction (Opute and Kadiri, 2013). Species colonization could also be due to increase in nutrient factors which directly increases growth of algae and reproduction (Opute and Kadiri, 2013). This could also be due to invasion and increase in algae density. These observations were obvious between 30th and the 40th day. The control experiment showed dense green colouration of the culture, this could be due to high density of algae arising from diversity of algae. However, this was not so in the treatment culture. Green colouration of the treatment culture was only obvious on the 20th day of the set up and this could be due to reduction in rate of reproduction of algal cell and their colonization of the culture by the reproductive algal cells. The growth of the various algal forms which in this study was observed as green colouration could have been occasioned by the addition or increase in the phosphate and nitrate content of the culture from the poultry dropping. This could have resulted in the overall stimulating and enhancing algal growth in both cultures. The additions of growth media in both samples enhance algal growth (Dubinsky *et al*., 1980). The non algal growth or non colonization of the treatment culture at the 10th day of the investigation could probably be due to high toxicity level of hydrocarbon at the onset of the study (Kori-Siakpere, 2000; Dunstan *et al*., 1975).

Algal composition and colonization pattern: The species colonization pattern observed in treatment culture could be due to the ability of the various forms to thrive at the experimented concentrations. This could also be due to hydrocarbon utilization(Takáčová *et al*., 2015).Hydrocarbon utilization is a process in natural system that brings about reduction in the hydrocarbon content of the environment and thus could be view as bioremediation process (Takáčová *et al*., 2015). Hydrocarbon affect growth, physiological process and reproduction in algae (Dunstan *et al*., 1975), this also could have affected the diversity of the various forms (Dunstan *et al*., 1975).The presence of algal species on the 20th day in the treatment culture could be due to evaporation and dilution which invariably has reduced the starting concentration and the toxicity of the culture (Roy and Mitra, 2011). It could also be due to acclimatization of the cells to the new environment (Takáčová *et al*., 2015). Also, it could be due to biodegradation of the toluene in the culture by the microalgae (Senngar *et al*., 2011). The colonization pattern in this study refers to the manner and nature in which the microalgae gradually colonize the culture. The algal composition in the treatment culture composed of different algal species in the division Cyanophyta, Euglenophyta, Chlorophyta and Desmids. The Cyanobacteria and the Euglenophytes were the most dominant species. The Cyanobacteria represented by *Oscillatoria, Lyngbya* and *chroococcus* were the pioneer species in the treatment culture. This could be due to the active role they play in bioremediation of toluene polluted environment. This finding corroborated with the work carried out by Takacova *et al*., (2015), the authors opined that algal selection and cultivation of choice species can be use to produce the oxygen required by acclimatizing bacteria to biodegrade hazardous pollutants such as polycyclic aromatic hydrocarbons, phenolics, and organic solvent.

6. CONCLUSSION

The degradation of pollutants 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.

SUMMARY OF RESEARCH

1.Toluene is a volatile compound belonging to the class monoaromatic hydrocarbon. It is a byproduct of crude oil that pollute the environment through 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.

2.The presence of different algal forms in the toluene polluted culture indicates the degradable potential of the various forms to toluene components and thus shows its significant role in its bioremediation.

3.In the colonization pattern of algal species in the treatment culture, Cyanobacteria were the pioneer species as they were first observed in the culture.

FUTURE ISSUES

Further research on biostimulation and bioaugmentation should be carried out in order to establish the best and efficient methods of optimizing the degradation potentials of the isolates. Cyanobacteria are photosynthetic and are believed to acquire carbon as a

source of energy from atmospheric CO2 and have been seen to degrade hydrocarbons. An investigation into whether it utilizes the carbon in the petroleum hydrocarbons and when, should be undertaken.

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