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Research Article

Toxicity of Water Accommodated Fractions of Waste Engine Oil on Growth of Selected Marine Algae

Yusuf O. Olaleye [1]* and Medina O. Kadiri [2]
[1] *Department of Marine Sciences, Faculty of Science, University of Lagos, Akoka – Yaba, Lagos, Nigeria*
[2] *Department of Plant Biology and Biotechnology, Faculty of Science, University of Benin, Benin, Nigeria*

*Corresponding author: yolaleye@unilag.edu.ng

ABSTRACT

Water Accommodated Fraction (WAF) is formed when water mix with oil under the influence of low or high energy. It is a solution of highly toxic mixtures of compounds which have high bioavailability to marine organisms and the potential of causing harmful effects. In this study, the growth inhibitory effects of WAF of waste engine oil on three (3) marine phytoplankton algae; *Isochrysis galbana*, *Thalassiosira pseudonana* and *Skeletonema tropicum* were investigated. The research was conducted in the laboratory for 14 days using different concentrations of WAF (5 %, 10 %, 15 %, 25 %, 50 %, 75 % and 100 %). Growth responses were measured spectrophotometrically at 750 nm absorbance. All the test algae were inhibited at concentrations ranging from 10 % to 100 % of WAF of waste engine oil whereas, at 5% concentration algal growth stimulation was observed. Similarly, there was a consistent decrease in dry weight of all the algae with increasing concentration of WAF of waste engine oil except at 5 %. *Isochrysis galbana* was generally more inhibited than *Thalassiosira pseudonana* and *Skeletonema tropicum*. There were significant differences ($p<0.05$) in growth of microalgae at various concentrations of the WAF of waste engine oil.

Keywords: Algae, Pollution, Waste engine oil, Water accommodated fraction
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INTRODUCTION

Marine phytoplankton are microscopic photosynthetic organisms, made up of an exceptionally diverse group of species that includes two domains of life, which are; domain eukarya and domain bacteria (Johnson & Martiny, 2015). They are responsible for primary productivity in the aquatic environment, thereby accounting for almost half of the global total production of oxygen (Field *et al*., 1998). Organisms at higher trophic levels depend directly or indirectly on phytoplankton for energy supply (Buskey *et al*., 2016). Therefore, any disturbance in phytoplankton population and/or alteration of primary production results to an adverse impact on other organisms in the environment (Cid *et al*., 2012).

Engine oil otherwise called lubricating oil is a petroleum product used mainly for friction reduction between engine surfaces. It contains chemical additives, which include heavy metals (Odjegba & Sadiq, 2002). Occasionally, oils are drained and replaced with new ones during engine servicing operation. The resulting spent oils are dark in colour due to carbon from wears, and are referred to as waste engine oils. Over 200 million litres of waste engine oils are generated in Nigeria annually, and are improperly disposed into the environment (Bamiro & Osibanjo, 2004). This incessant mode of disposal of waste oils has been linked to pollution incidents in the environments (Odjegba & Sadiq, 2002; Chukwu & Odunzeh, 2006).

Water Accommodated Fraction (WAF) is formed when water mixes with oil under the influence of low or high energy. It is a solution of highly toxic mixtures of compounds which are of special interest, as studies have shown these compounds have high bio-availability rate to marine organisms and potential of causing harmful effects (McAuliffe, 1987; Neff & Stubblefield, 1995; Hokstad *et al*., 1998; Santanda-Avancena *et al*., 2016). However, the influence of WAF of waste engine oil on marine phytoplankton

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algae has not been given proper attention. Consequently, the available literature is very scanty.

Previous works include Bott and Rogenmuser (1978) on the effects of water-soluble extracts of No. 2 fuel oil, Nigerian crude oil and used crankcase oil on attached phytoplankton community. The study reported that water-soluble extracts of No. 2 fuel oil and used crankcase oil exerted inhibitory effects on algal biomass (chlorophyll *a*), influenced blue-green algae dominance and decreased diatom population. Akpan and Frank (2003) investigated the effects of waste engine oil on the phytoplankton of Calabar River Estuary, and observed growth depression in diatoms population, however, growth stimulation was reported in nanoflagellate species. Etim and Okon (2011) studied the effects of diesel oil on phytoplankton species and observed growth inducement in *Coscinodiscus excentricus*. Kadiri and Eboigbodin (2012) examined the effects of water-soluble fractions (WSF) of refined petroleum products on microalgae and reported that WSF of fuel oils were stimulatory at low concentrations of diesel and kerosene, whereas, at high concentrations, growth inhibition was observed and the reported order of toxicity were kerosene > petrol > diesel.

Pollution caused by waste engine oil is an endemic problem in Nigeria, and it is more prevalent than crude oil pollution. Therefore, the objectives of this study are: to assess the growth responses and toxicity of the selected marine algae to WAF of waste engine oil.

MATERIALS AND METHODS

Test Microalgae and Culture Conditions

Pure strains of coccolithophore (*Isochrysis galbana*) and diatoms (*Thalassiosira pseudonana*, and *Skeletonema tropicum*) were used in this study. They were collected off Lagos coast (Bar beach) and grown in an F/2 medium as described by Guillard and Ryther (1962). Isolation and subsequent sub-culturing of samples to obtain unialgal culture were done using serial dilution and agar plating techniques. Florescent bulbs were used as source of illumination and all algal samples were lightly aerated to avoid clumping of cells. Proper identification of species was done using relevant texts (Hasle *et al*., 1996; Tomas, 1997).

Preparation of Water Accommodated Fraction (WAF) of Waste Engine Oil

Waste engine oils were collected from an automobile servicing station in Yaba, Lagos, Nigeria. The water accommodated fraction (WAF) was prepared according to the method of Karam *et al.* (2014) using a single oil: water (1:10) loading; the oil was layered gently from the top over the water and stirred for 48hrs using INTLLAB Magnetic stirrer (3000 mL capacity). Thereafter, the oil-water mixture was allowed to stand overnight in separating funnel, covered with black cellophane to prevent photo-oxidation.

Experimental Set-up

Toxicity test using introduced micoalgae was conducted following the International Organization for Standardization guideline (ISO, 2016). The aqueous fraction of WAF was considered as 100 % solution and was diluted with the prepared F/2 medium to give 75, 50, 25, 15, 10, 5 and 0 % WAF of waste engine oil which were stored at room temperature (25 ℃) in transparent plastic bottles (250 mL - final volume) with screw-cap prior to inoculation of the test algae.

Each test microalga was exposed separately to all the different concentrations of WAF from waste engine oil. A control experiment was also conducted where the test organisms were exposed to the same conditions using F/2 medium only (0 % WAF). All tests were conducted in three replicates. The pH, total dissolved solids (TDS), conductivity and salinity were monitored regularly. The growth of microalgae was measured optically using a spectrophotometer (HACH DR/2000 Direct reading spectrophotometer) at 750nm every 2 days for 14 days.

Dry Weight Estimation

The dry weight of the test algae was estimated every two days using the formula described by Horvatic *et al.* (2003).

Dry weight =

$$3.31 + 179.45 \text{ x Absorbance @ } 750nm + 617.45 + (\text{absorbance @ 750 nm})^2$$

Percentage Inhibition

The percentage inhibition was calculated for the test alga on day 12 of the experiment using the formula:

$$\text{Percentage inhibition} = 100 - \frac{\text{Measured biomass}}{\text{Theoretical biomass}} \times \frac{100}{1}$$

(Phatarpekar & Ansari, 2000).

Where: Measured biomass = Growth of test alga in other treatment

Theoretical biomass = Growth of test alga in control

Statistical Analysis
All data obtained were subjected to ANOVA. Test of significance was conducted and effects were considered significant if ($p < 0.05$). Further test of significance (post-hoc analysis) was done using Duncan Multiple Range test (DMRT) (SPSS statistics version 22).

RESULTS

The growth response of *Isochrysis galbana* in WAF of waste engine oil is represented in Figure 1. The result showed a consistent decrease in growth with increasing concentrations of WAF. Its growth at 5% is at par with the control. The maximum growth was recorded in 5%, followed by 10%. At these concentrations, growth increased consistently, reaching maximum on day 12 and declined thereafter. Other concentrations recorded minimal or no growth. Growth at 100% was severely inhibited. Statistically, there was a strong significant difference ($p < 0.05$) in the growth of *Isochrysis galbana* in the various concentrations of waste engine oil. Post hoc analyses using DMRT also revealed that 0 % and 5 % concentrations of WAF of waste engine oil had the highest mean values, followed by 10 % WAF of waste engine oil.

The Growth pattern of *Thalassiosira pseudonana* in WAF of waste engine oil is presented in Figure 2 where growth at 5 % was noticeably higher than control, signifying stimulation. Growth consistently increased from day 0 to day 2 in all the various concentrations. The maximum growth was recorded in 5 %, followed at a distant second by 10 %. Statistical analysis of the obtained data showed strong significant differences ($p < 0.05$) in the growth of *Thalassiosira pseudonana* in various concentrations of waste engine oil. Post hoc analyses using DMRT revealed that 5 % and 0 % had the highest mean values, followed by 10 % and 15 % WAF of waste engine oil.

The influence of WAF of waste engine oil on the growth of *Skeletonema tropicum* is expressed in Figure 3 where growth at 5 % was higher than control thereby signifying stimulation. A lag phase was noticed up to day 2 in most of the concentrations. The set of data were analyzed at a 0.05 level of significance. The result showed significant differences in the growth of *Skeletonema tropicum* in the various concentrations of waste engine oil $p < 0.05$. Post hoc analyses using *DMR* test also revealed that 5 % and 0 % had the highest mean values, followed by 10 % WAF of waste engine oil.

The percentage inhibition of the three test marine phytoplankton algae in WAF of waste engine oil is portrayed in Figure 4. *Isochrysis galbana* had the highest inhibition relative to the two other microalgae. The differential inhibition of *Thalassiosira pseudonana* and *Skeletonema tropicum* was insignificant.

The dry weights of *Isochrysis galbana*, *Thalassiosira pseudonana* and *Skeletonema tropicum* are illustrated in Figure 5. Generally, there was a consistent decrease in dry weight with increasing concentration of WAF except at 5% where growth stimulation occurred.

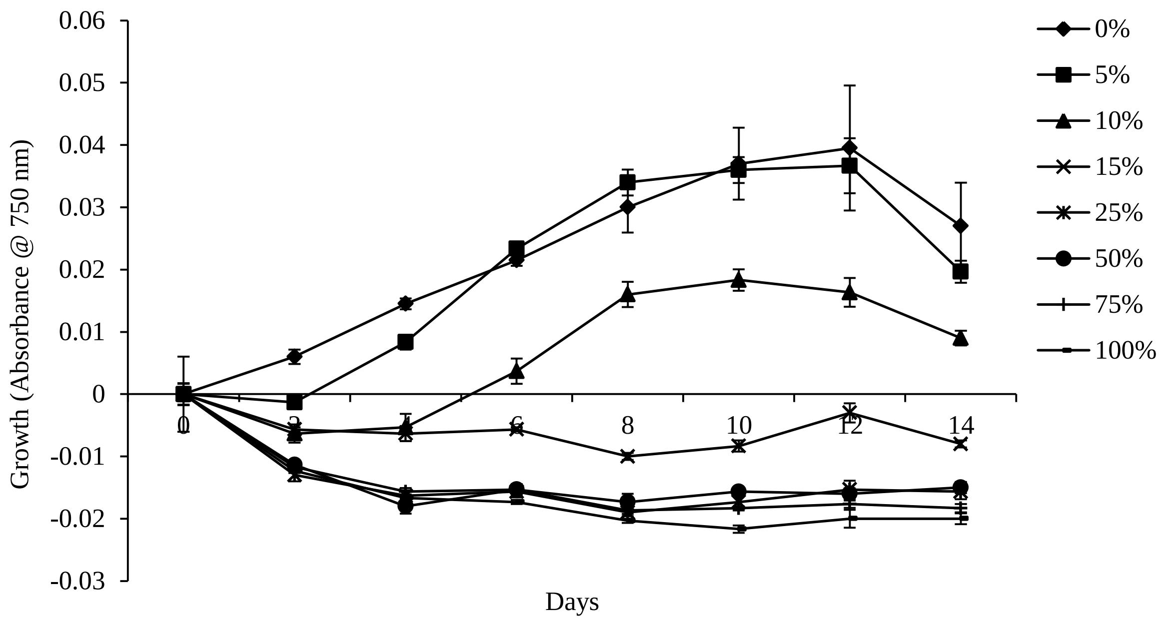

Figure 1: Growth response of *Isochrysis galbana* in WAF of waste engine oil

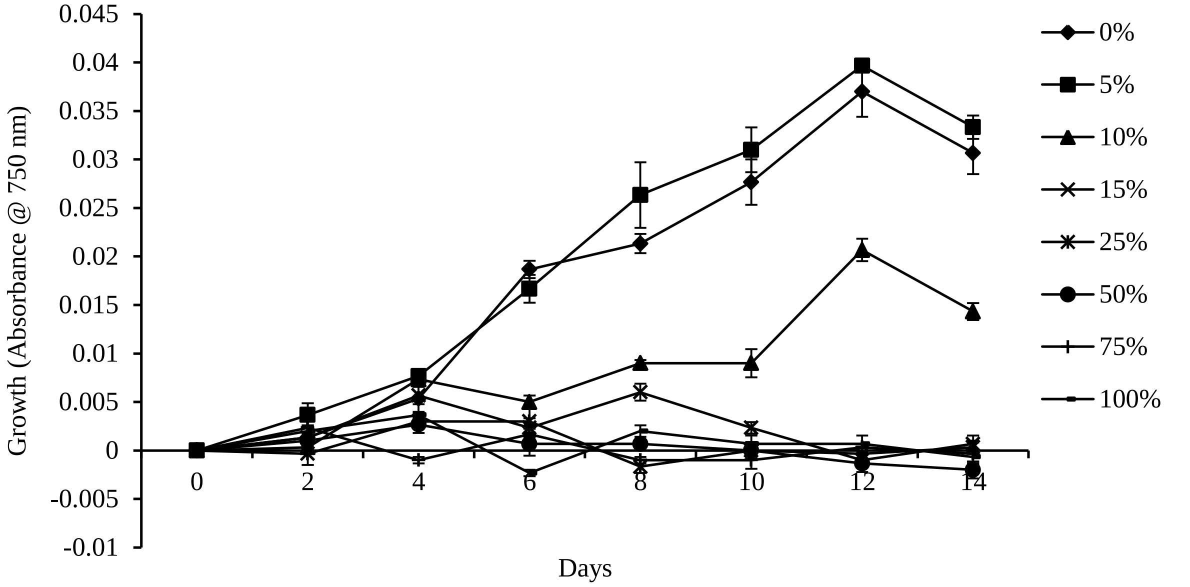

Figure 2: Growth response of *Thalassiosira pseudonana* in WAF of waste engine oil

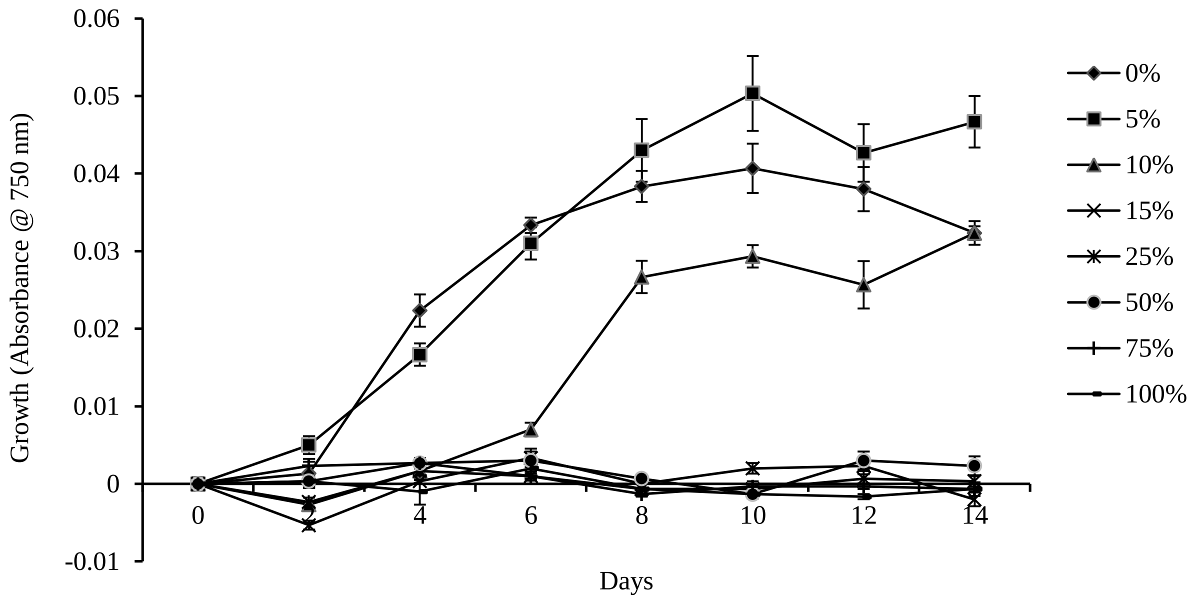

Figure 3: Growth response of *Skeletonema tropicum* in WAF of waste engine oil

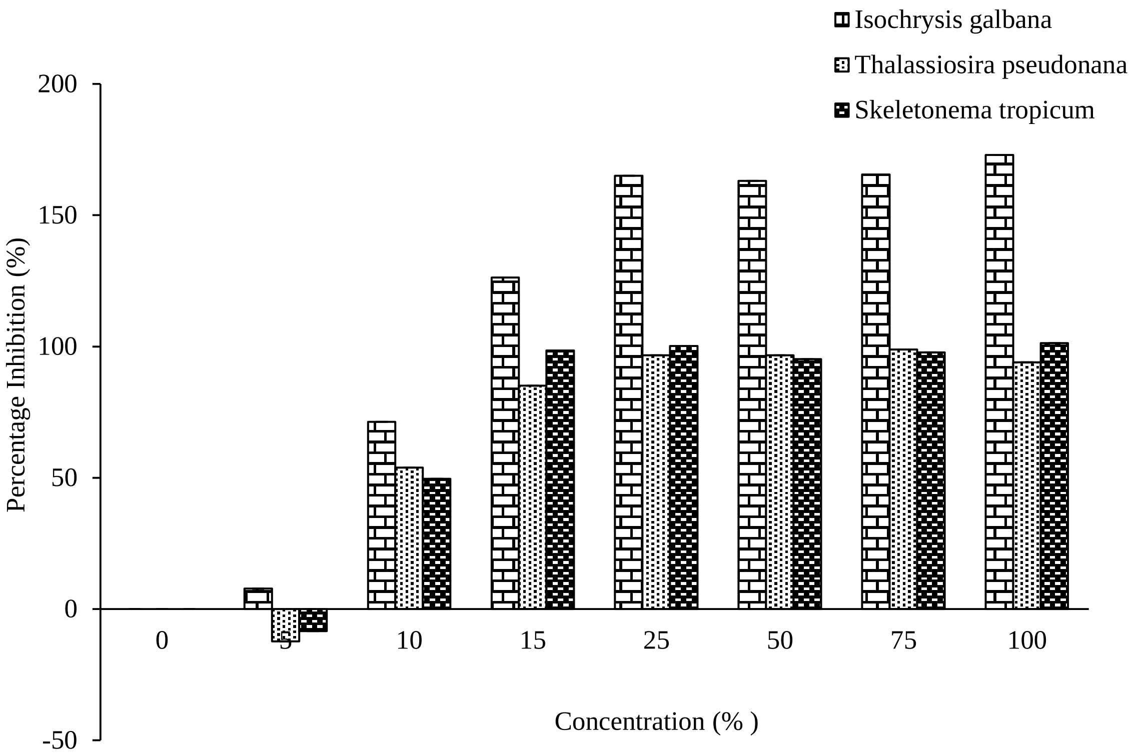

Figure 4: Percentage inhibition of the test algae in WAF of waste engine oil

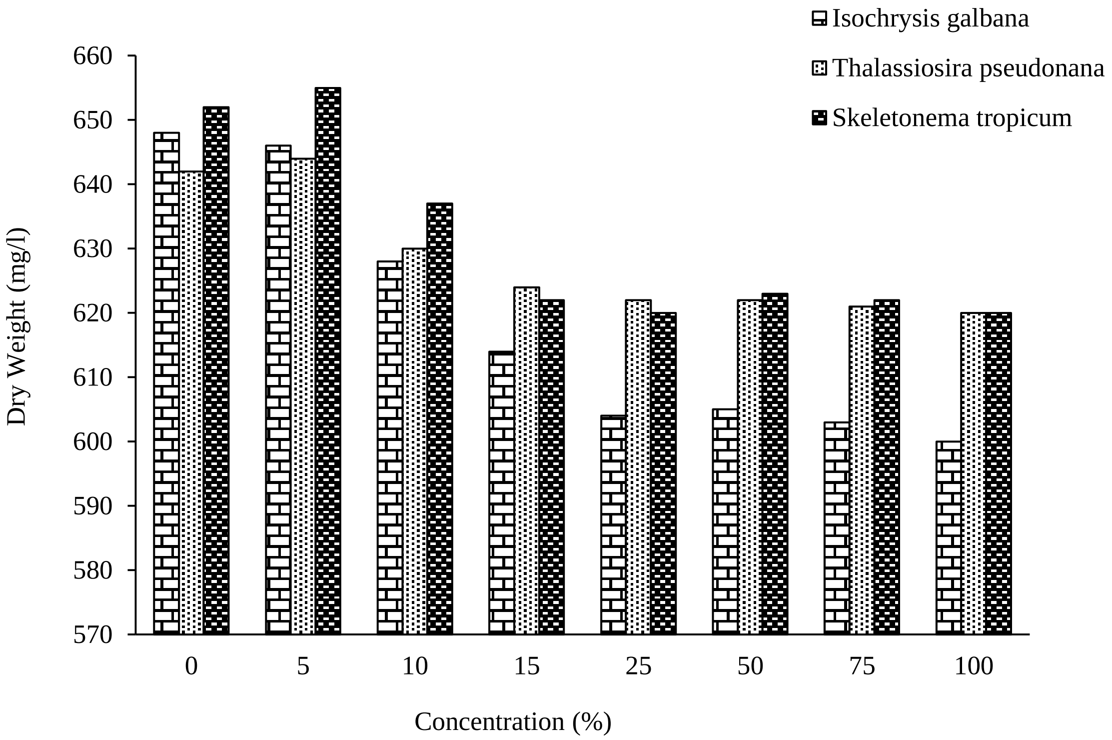

Figure 5: Dry weights of the test algae in WAF of waste engine oil

DISCUSSION

The results obtained in this study showed variability in the sensitivity of different algal species to WAF of waste engine oil. Microalgae cells were affected remarkably at 10 %, 15 %, 25 %, 50 %, 75 % and 100 % concentrations. However, the growth performance of the untreated algae species (control) was similar in all cultures, but after the WAF was added, microalgae cells decreased in all treatments except at 5% where growth stimulation occurred. The consistent decrease in growth of algae with increasing concentrations of WAF in this study agreed with the report of Kadiri and Eboigbodun (2012) that growth retardation occurs at higher concentrations of Water Soluble Fraction (WSF) of kerosene, petrol, and diesel.

Also, growth stimulation recorded at 5 % concentration conformed to the studies of El-Sheekh *et al.* (2000), who reported the stimulatory influence of low concentration of crude oil and its refined products on growth and metabolic activities of freshwater algae, *Chlorella homosphaera* and *C. vulgari*. The result is also in consonant with the studies of Kadiri and Eboigbodun (2012) who reported that at low concentrations (10 - 25 %) of water soluble fraction (WSF) of diesel and kerosene, *Desmodesmus quadricauda* experienced growth stimulation.

The ability of the microalgae to utilize the water accommodated fraction of the engine oil as a source of carbon for photosynthesis explained the reasons why growth stimulation occurred at low concentrations (El-Sheekh *et al.*, 2000; Parab *et al.*, 2008). Other justifications for growth stimulation according to Bott and Rogenmuser (1978) include volatilization and adaptation of the algal population to the hydrocarbon. Higher concentrations, on the other hand, caused growth depression and inhibition resulting from high levels of lethal component of fuel oils. The implication of high concentration of fuel oil was also reported by El-Dib *et al.* (2001) who stated that the carbohydrate content of algal cells decreased significantly as a result of high concentrations of fuel oils.

In this study, *Isochrysis galbana* seem to be more susceptible to engine oil. The algae with toxicity tolerance to waste engine oil WAF was *Skeletonema tropicum* followed by *Thalassiosira pseudonana*. The differential response of the distinct algae to the fuels may be due to the differences in the cell wall compositions of species or a presence of a native physiological and chemical defence system (Podkuiko, 2013).

This study also supports the supposition that variation in responses of marine phytoplankton algae to oil pollution often results to a non- normal species succession, whereby the species with high tolerant threshold becomes the dominant group (Huang *et al.*, 2011). Consequently, alteration of microalgae communities leads to change in zooplankton communities resulting from a selective mode of feeding (Perez *et al*., 2010) and by extension affecting other marine animals.

Since *Skeletonema tropicum* and *Thalassiosira pseudonana* were tolerant to the waste engine oil, these phytoplankton algal species could be considered as potential candidates for bioremediation of waste oil. This study revealed that WAF of waste engine oil at 5 % concentration enhanced algal growth while, other concentrations higher than 5 % inhibited algal growth.

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