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Response of Four Phytoplankton Species Found in Some Sectors of Nigerian Coastal Waters to Crude Oil in Controlled Ecosystem

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ABSTRACT: Identification and enumeration of phytoplankton species from Ilaje and Lagos sectors of the Nigerian coastal waters were conducted using standard procedures. Effects of different crude oil concentrations (6 to 50 ppm) on population of *Coscnodiscus centralis, Thalassionema frauenfeldii, Odontella mobiliensis*, and *Ceratium trichoceros* at different exposure periods (6 to 42 h) via microcosm experiments were then assessed. Results showed that the phytoplankton species consisted of diatoms (83.33%) and dinoflagellates (16.67%) whose abundance ranged from 2 to 516 Cell/mL. Crude oil toxicity varied from 0.06 to 36.43% for *C.centralis*, 1.41 to 35.58% for *C.trichoceros*, 1.71 to 46.11% for *T.frauenfeldii* and 0.66 to 44.90% for *O.mobiliensis* and showed direct relationship (r -+ 0.81 to +0.97; $p < 0.001$) with concentration but inverse with exposure period (r = -0.83 to – 0.90; $p < 0.001$). Vulnerability within 24-h contact decreased in the order: *T.frauenfeldii* > *O.mobiliensis* > *C.centralis* > *C.trichoceros*. Study is a contribution to the scarce data bank on crude oil dose-response assessment on plankton species in Nigeria, demonstrating that influx of crude oil into the Nigerian coastal waters is a risk factor to ecological status.

Key words: Crude oil, Coastal waters, Phytoplankton, Toxicity, Environment, Nigeria

INTRODUCTION

Nigeria is one of the leading oil producing countries of the world with an economy heavily dependent on the oil sector, which provides about 20% of gross domestic product, 70 to 90% of foreign exchange earnings and about 65% of budgetary revenues. Crude oil, for several decades, has been the main export product of Nigeria and the oil reserve is put at 36 billion barrels while production is estimated at 2.1 million barrels per day (Eyong *et al.*, 2004; Egberongbe *et al.*, 2006; Adeniyi *et al.*, 2008). The oil reserves are found in relatively simple geological structures along the country's coastal Niger Delta and deeper water offshore.

The Niger delta region consists of the states of Abia, Akwa Ibom, Bayelsa, Cross River, Delta, Edo, Imo and Ondo, all in the southern region of the country. Typical oil wells, pipelines and infrastructure in the Niger Delta are shown in Fig.1.

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According to available statistics, in the last 30 years more than 400,000 tons of oil has spilled into the creeks and soils of southern Nigeria (Egberongbe *et al.*, 2006). Oil spills in Nigeria occur due to a number of factors including sabotage, corrosion of pipes and storage tanks, carelessness during production operations and oil tankers' accidents. Oil obviously has brought economic boost to the country but the exploration activities, associated spills in the Niger Delta and attendant environmental issues have been of national and global concern (Powel *et al.*, 1985). Reports on the negative environmental impact ranging from vegetation destruction, water pollution, destruction of some terrestrial and aquatic flora and fauna are documented (Isichei and Sanford, 1979; Powel *et al.*, 1985; Kakulu and Osibanjo, 1986; Snowden and Ekweozor, 1987; Kakulu *et al.*, 1987; Kakulu and Osibanjo, 1988; Adekambi, 1989; Jinadu, 1989; Eyong *et al.*, 2004; Igwebuike *et al.*, 2007).

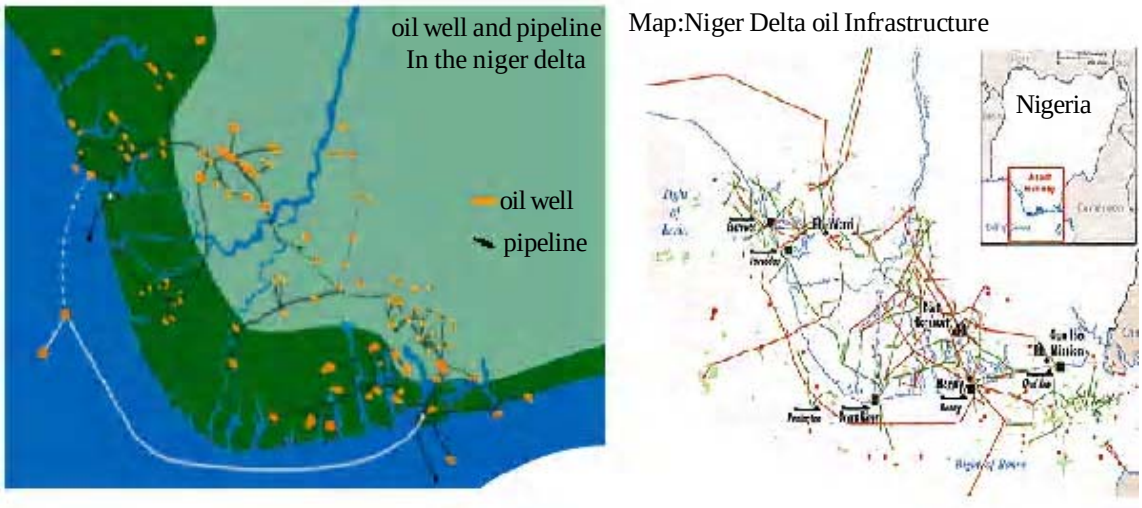

Fig. 1. Some oil wells and oil infrastructure in the Niger Delta

Studies on crude oil effects on phytoplankton species, which are not just aquatic primary producers, the base of food web structure but also a key factor to global oxygen production in the country, are limited. Previous studies on phytoplankton research in Nigeria were primarily focused on composition, abundance and temporal variation (Khan and Ejike, 1984; Opute, 1990; Kadiri, 1993; Kemdirim, 2001; Ezra and Nwankwo, 2001; Ajayi and Akonai, 2003; Akin-Oriola, 2003; Chindah, 2003; Chindah, 2004; Nyananyo et al., 2006; Essien *et al.*, 2006; Essien *et al.*, 2007; Akinyemi and Nwankwo, 2007; Ajayi *et al.*, 2007). Ekom (2006) investigated the effect of waste engine oil on phytoplankton of the Calabar river estuary while Obire and Anyawu (2009) assessed the effect of various concentrations of crude oil on fungi populations of soil. Identification of microalgal bioindicator for crude oil pollution and natural remediation as well as petroleum hydrocarbon levels in some organisms and sediments from Qua Iboa estuary, Nigeria were also investigated (Essien *et al.*, 2006; Essien *et al.*, 2008; Benson *et al.*, 2008).

Although several decades of research has produced data on oil and hydrocarbon effects on a wide range of phytoplankton species in the developed countries (Gordon and Prouse, 1973; Miller *et al.*, 1978; Kusk, 1980; Karydis and Fogg, 1980; Kelly *et al.*, 1999; Dzierzbicka-Glowacka, 2007), there is paucity of information on risk assessment with regards to a defined dose-response relationship for Nigerian crude oil to species of phytoplankton found in the Nigerian coastal waters.

Experiments for optimization purposes are better carried out in controlled environments rather than open ecosystems such as the marines due to the fact that species like phytoplankton are passively carried along by water currents and their biomass is affected by processes such as seasonal variation, turbulent diffusion, sinking, primary production, respiration, natural mortality, grazing, cell lyses or sudden appearance of in the habitat, toxic pollutant other than the investigated xenobiotic chemical (Kelly *et al.*, 1999; Onyema, 2007; Adekunle *et al.*, 2007a; Dzierzbicka-Glowacka, 2007).

This study was therefore designed to assess the risk of low level crude oil concentrations on the population of four phytoplankton species (*Thalassionema frauenfeldii, Cosnodiscus centralis, Odontella mobiliensis and Ceratium trichoceros)* found in the costal waters of Ilaje, Ondo state (oil producing area) and Bar-beach Lagos (non-oil producing area) under controlled laboratory conditions.

MATERIALS & METHODS

Ilaje is located in Ondo state, Niger Delta region of Nigeria and comprises many towns including the study locations namely Aiyetoro, Ilowo and Ilepete communities, located along the shore line (Fig. 2).

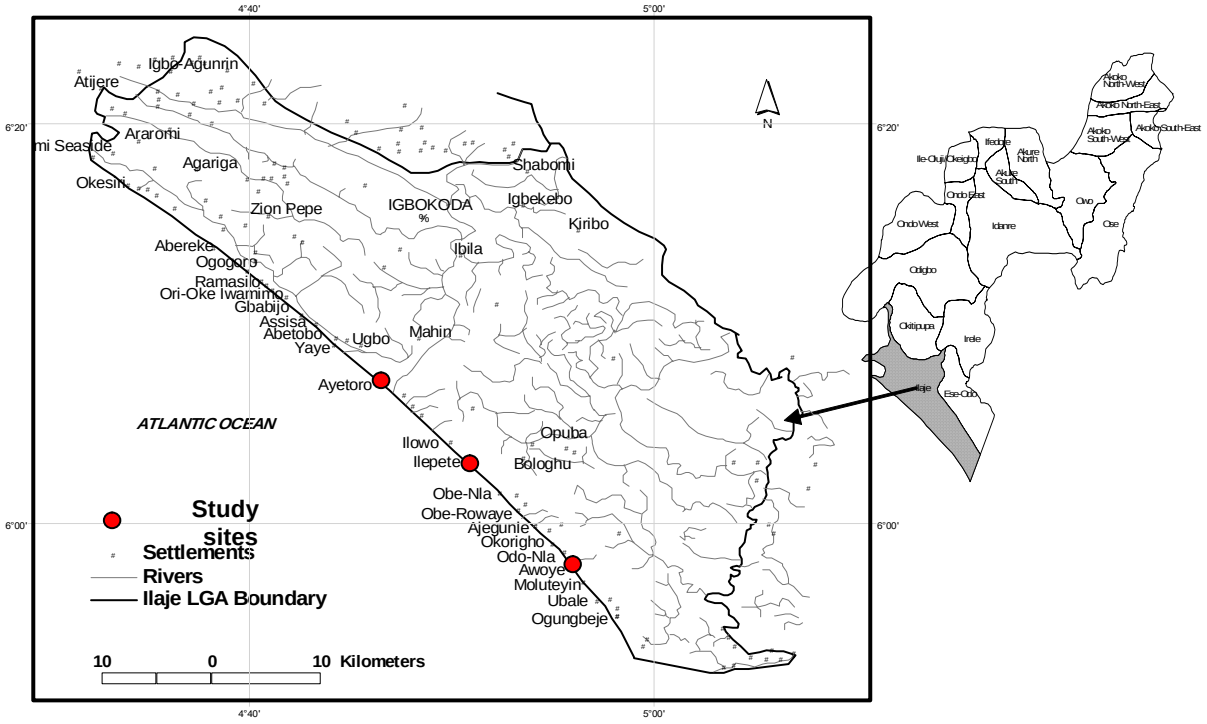

Fig. 2. Ilaje communities in Ondo state showing Ayetoro, Ilepete and Awoye

The area is characterized by a difficult terrain owing to the predominantly shoreline/marine nature, silt, mud and sedimentary deposits, freshwater swamps and marshland with few islands in-between. Bar-beach Lagos is one of the two popular beaches in Lagos, Nigeria, selected as a coastal segment found in non-oil producing area of the country.

The physico-chemical characteristics of sea water samples were determined following standard procedures (APHA, 1989; Adekunle et al., 2007b). Temperature, salinity, pH and electrical conductivity were measured using 0 – 100°C mercury in glass thermometer, and HANNA series of meters. The pH meter was calibrated with 4 and 9 buffers. Phosphate was analyzed as orthophosphate with the ammonium molybdate - ascorbic acid spectrophotometric procedure. The resulting blue colored antimony-phospho-molybdate complex was measured at 880 nm using a 1 cm ë 3B Perkin Elmer spectrophotometer. A standard calibration curve was prepared using potassium hydrogen phosphate salt (KH_2PO_4) in the range of 0.05 to 0.35 mg/L.

Nitrate was determined via the phenoldisulphonic method. The absorbance values were recorded on the same spectrophotometer but at the wavelength of 410 nm and a calibration curve was prepared from KNO_3 whose working range varied from 0 to 3 mg/L. Dissolved oxygen was determined by direct probe analysis using dissolved oxygen meter. Biochemical oxygen demand (BOD) was assessed via dilution method and chemical oxygen demand (COD) by the dichromate procedure under reflux.

Phytoplankton samples were collected from Aiyetoro, Ilowo and Ilepete in Ilaje, Ondo State and Bar-beach Lagos, Lagos State, Nigeria using 53ìm plankton net and 1.5 L specimen plastic bottles. With the aid of a fishing boat, the net was lowered into the seawater, 5 cm deep, and dragged for five minutes while the boat was still in motion. Only short tows of 5 minutes were taken to reduce error caused by net clogging. The tow replicates were taken at different times of the day at each study station. Each sample was concentrated to a volume of 200 mL. The plankton samples for species identification and enumeration were transferred into sampling bottles and fixed with 5% formalin. Those for toxicity test were transferred into sampling bottles that contained no formalin so as to keep alive. At the chemical

laboratory, phytoplankton species were enumerated using a 1 mL –Sedwick-Rafter counting chamber and each was counted 3 times on a phase contrast compound microscope with an eye piece. The common marine plankton identification key was utilized (Kolb, 1986; Kemdirim, 2001).

Nigerian crude oil with specific gravity (AP) of 41.7 to 43.0 was used and experiment was carried out under illumination (12:12 h light: dark period) provided by white fluorescent lamps of 20 W each at room temperature of 29± 1°C . A stock solution (1000 ppm) of the crude oil was prepared by taking the equivalent volume that gave 0.1g crude oil using a micropipette, which was then transferred to a 100 mL volumetric flask, already containing 30 mL of n-hexane and made up to mark with the solvent. Seven different lower concentrations of crude oil (6, 8, 10, 14, 20 and 50 ppm) were thereafter prepared from the stock solution and the effect of each crude oil concentration on the population of the 4 phytoplankton species (*T. frauenfeldii*, *C.centralis, O.mobiliensis* and *C.trichoceros*) at different contact periods of 6-h interval (6, 12, 18, 30, 36 and 42 hours) was investigated as subsequently described.

Erlenmeyer 500 mL flasks were used as incubation reactors and triplicates of each treatment and control (consisting only of n-hexane treatments to the phytoplankton species) were set up. Exactly 200 mL sea water was filtered through a 0.22 ìm membrane and the initial population of each of the 4 phytoplankton species recorded as previously described. A given concentration of crude oil (6, 8, 10, 14, 20 or 50 ppm) was gradually added and the flask gently swirled for uniform distribution and aerators were connected to all the flasks. The reduction in phytoplankton population (mortality) was used as a measure of response to crude oil induced stress; hence, phytoplankton species cell abundance at the end of each experiment was related to the initial population and expressed as percentage mortality:

$$\text{Mortality (\%)} = [P_i - P_f] - [P_i - P_c]/ P_i \times 100$$

where P_i = initial cell abundance, P_f = cell abundance at the end of the crude oil treatment and P_c = cell abundance at the end of the control experiment.Data generated, generally expressed as mean ± standard error, were subjected to descriptive statistics, one way analysis of variance (ANOVA) and Pearson correlation analyses using SPSS 10.0 for Windows to establish significant variations and relationships.

RESULTS & DISCUSSION

The physico-chemical parameters of the seawater at the different study locations (Table 1) varied from 1.23 to 2.01 mg/L for NO_3^-, 0.08 to 0.20 mg/L for PO_4^{3-}, 31 to 33‰ for salinity, 6.36 to 7.35 for pH, 49.0 to 61.1 mg/ L for COD, 29.0 to 31.5 mg/ L for BOD and 3.1 to 5.9 mg/ L for DO.

The identified phytoplankton species, whose relative abundance ranged from 2 to 516 Cell/mL, consisted of 83.33% diatoms and 16.67% dinoflagellates. They were diatoms *Coscnodiscus centralis, Asterionellopis glacialis, Thalassionema frauenfeldii, Odontella mobiliensis, Chaetocero mitra, Rhizosolenia imbricate, Bacellaria paxillifera, Cerataulina palegica, Navicula spp, Pleurosigma spp* and dinoflagellates *Ditylum spp and Ceratium trichoceros.*

Phytoplankton mortality due to crude oil (Figs. 3 and 4) increased with concentration of oil irrespective of the exposure period. Generally, crude oil toxicity on the population varied from 0.06 to 36.43% for *C.centralis*, 1.41 to 35.58% for *C.trichoceros*, 1.71 to 46.11% for *T.frauenfeldii* and 0.66 to 44.90% for *O.mobiliensis*. Results revealed that the resistance to crude oil toxicity varied with phytoplankton species. For instance, Fig. 3 showed that at 6 h exposure, the toxicity decreased as follows: *T.frauenfeldii* > *O.mobiliensis* > *C. trichoceros* > *C.centralis*. However, at 12 h exposure, *C.centralis* exchanged position with *C.trichoceros* (*T.frauenfeldii* > *O.mobiliensis* > *C.centralis* > *C. trichoceros*). The same pattern was observed at 18 h contact period. Unfortunately, *C.trichocerus* did not survive beyond this time so became extinct due to natural mortality. Beyond 30 h, *O.mobiliensis* also became extinct and *T frauenfeldii* could not survive beyond 36 hours so also became naturally extinct (Fig.4). leaving *C.centralis* as the only

Table 1. Physicochemical characteristics of the seawater, phytoplankton species composition and abundance at the study locations

Variable	Study location			
	Awoye	Ayetoro	Ilepete	Bar-Beach
Physicochemical parameters				
pH	7.19 ± 0.2	7.35 ± 0.01	7.35 ± 0.04	6.36± 0.01
Temperature (^oC)	33±1	33± 1	33±1	30±1
Salinity (‰)	31 ± 1	33± 1	33 ± 1	31± 2
Nitrate (mg/L)	1.23± 0.01	1.65± 0.02	2.01±0.01	1.77±0.01
Phosphate (mg/L)	0.08 ± 0.01	0.18 0.03	0.10 ±0.01	0.20±
Electrical conductivity (mS/cm)	48.3± 0.2	52.0 0.1	52.0 ±0.3	48.8 ±0.2
Biochemical oxygen demand (mg/L)	31.5±0.4	29.0±0.1	28.3±0.2	29.9±0.3
Chemical oxygen demand (mg/L)	61.1± 0.5	57±0.2	53±0.2	49±0.4
Dissolved oxygen (mg/L)	3.1±0.1	3.7±0.1	3.6±0.2	5.9±0.1
Diatom (Cell/L)				
Coscnodiscus centralis	4.68 ± 1.0	3.14 ± 1.3	3.31 ± 0.1	5.16 ± 1.3
Asterionellopsis glacialis	1.37± 0.3	5.4 ± 0.3	2.2 ± 0.2	2.9 ± 4
Thalassionema frauenfeldii	1.62 ± 1.0	1.13 ± 0.2	9.6± 0.9	1.58 ± 0.6
Odontella mobiliensis	2.13 ±0. 7	1.76 ± 1.5	2.0±0.1	1.1 ± 0.3
Chaetoceros mitra	9.6 ± 0.2	2.7 ± 0.2	3.6± 0.1	9.0 ± 0.5
Rhizosolenia imbricate	1.3± 0.1	8.0 ± 0.1	1.9± 0.1	2.9 ± 0.6
Bacellaria paxillifera	2.8 ±0.5	2.0 ± 0.1	1.7 ± 0.2	6.2 ± 0.7
Cerataulina palegica	1.66± 1.3	1.73 ± 1.2	1.6± 0.9	Nil
Navicula species	7.0± 1.0	6.0 ± 0.1	1.3 ± 0.6	1.6 ± 0.4
Pleurosigma species	8.0± 1.0	1.3 ± 0.1	1.6 ± 0.6	1.5 ± 0.3
Dinoflagellate (Cell/L)				
Ditylum species	5.1± 2	4.1 ± 1	4.9 ± 1	4.9 ± 0.5
Ceratium trichceros	5.4 ± 2	3.0±1	7.0 ± 1	1.14 ± 0.2

surviving organism at 42 h - contact with crude oil. Fig. 3 showed that of the four, *T. frauenfeldii* was the most vulnerable to crude oil toxicity within 24 - h contact.

In contrast to the increasing toxicity trend observed with regards to crude oil concentration, toxicity decreased with exposure period for each of the species (Fig.5). The relationships between the crude oil induced toxicity and the two variables (oil concentration and exposure period) showed high and significant correlations, with coefficients in the range of + 0.81 to + 0.97 ($p < 0.01$) for oil concentration and – 0.83 to – 0.90 ($p < 0.01$) for exposure period.

The physico-chemical parameters of the seawater used in this study were generally suitable for supporting lives in the aquatic ecosystem (Chindah, 2003). The dominance by diatoms (Order: Bacillariophyceae) was in line with the report of Wang *et al*. (2004) who stated that diatoms are a major group of eukaryotic algae and are one of the most common types of phytoplankton, mostly unicellular, green in color, impacting green coloration to water and have a shell of silicon (Kolb, 1986). From previous Nigerian works, notable among phytoplankton found in the aquatic systems were Bacillariophyta, Chlorophyta, cyanophyta, Dinophyceae and Euglenophyta (Opute, 1990; Kadiri, 1993; Kemdirim,, 2001; Ezra and Nwankwo, 2001; Ajayi and Akonai, 2003; Akin-Oriola, 2003; Chindah, 2003; Chindah, 2004; Nyananyo *et al.*, 2006; Essien *et al.*, 2006; Onyema, 2007; Akinyemi and Nwankwo, 2007; Essien *et al.*, 2007; Davies *et al.*, 2008).

The adverse impact of low level crude oil concentrations (6 to 50 mg/L) on the phytoplankton population and biodiversity showed toxicological effect of crude oil to these organisms, which are very sensitive ecological indicators, thereby

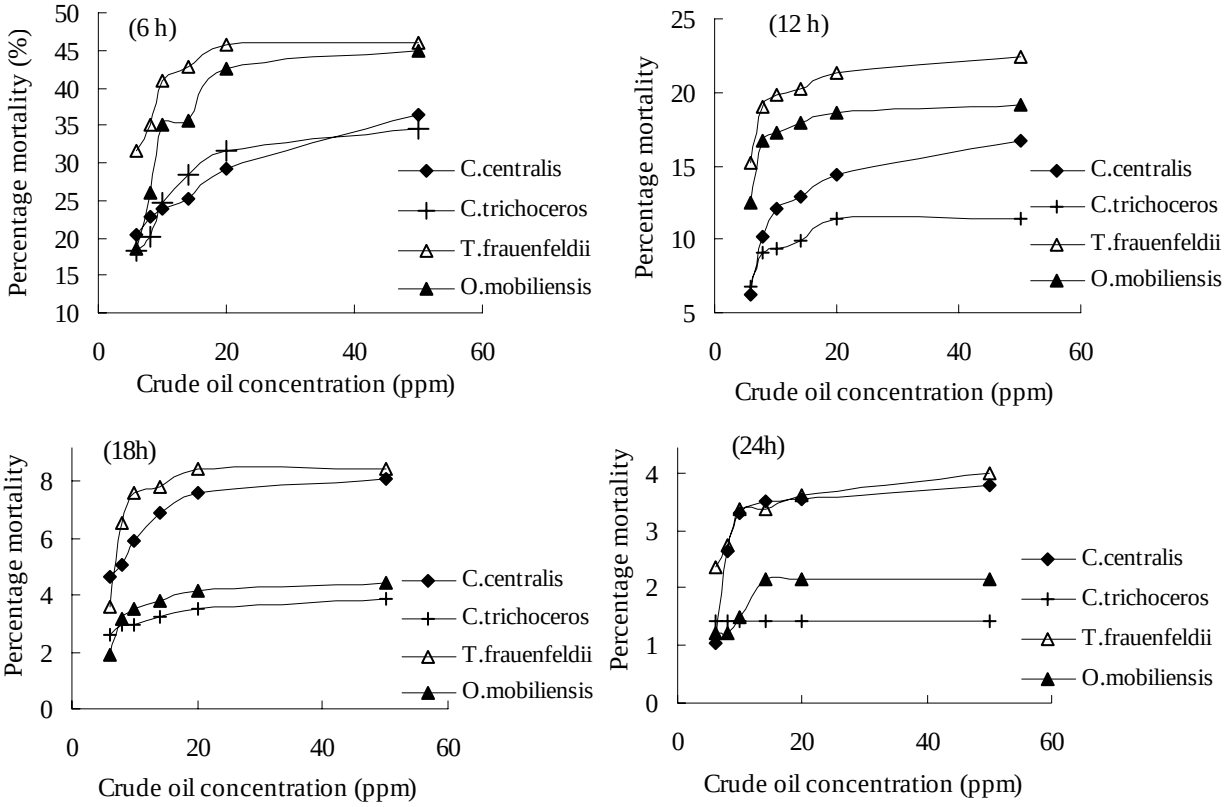

Fig. 3. Percentage toxicity of crude oil on population of C.centralis, C.trchoceros, T.frauenfeldii and O.mobiliensis as a function of concentration at a given exposure period

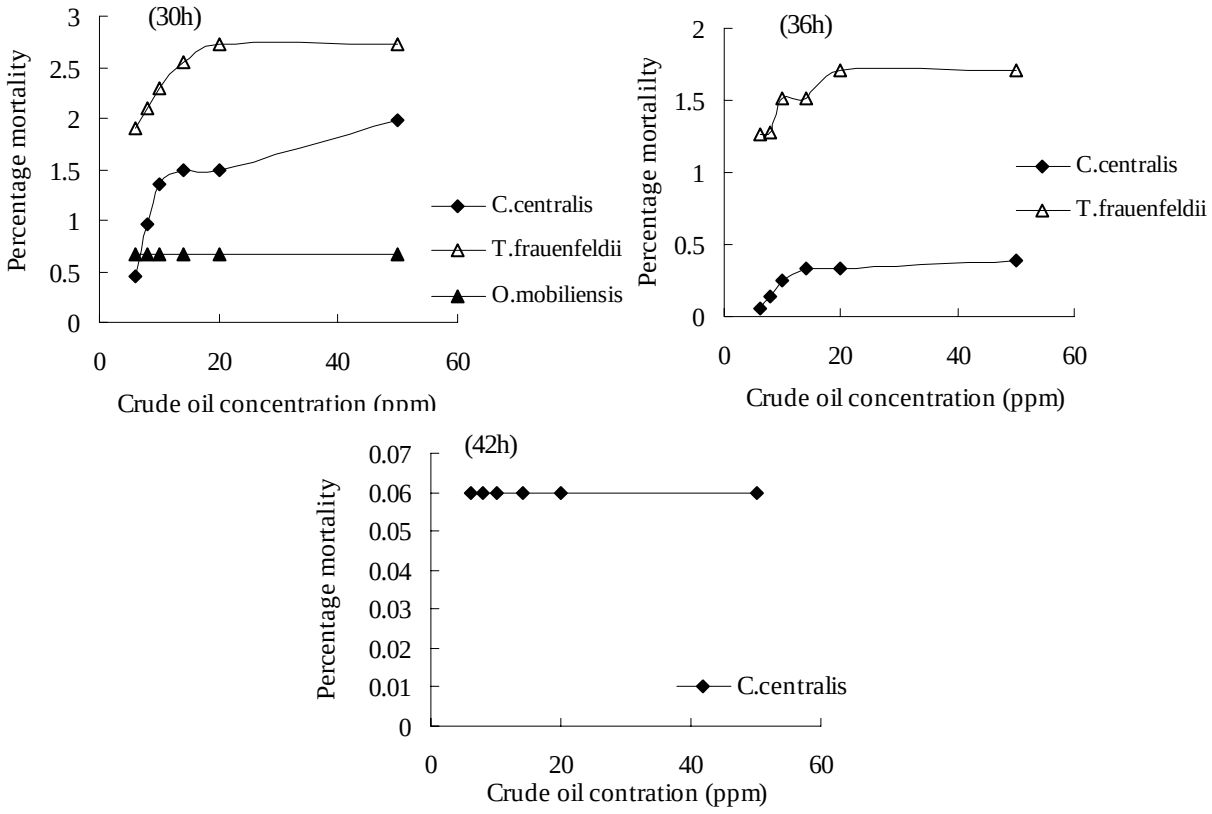

Fig. 4. Percentage toxicity of crude oil on population of C.centralis, C.trchoceros, T.frauenfeldii and O.mobiliensis at 30, 36 and 42 hour exposure periods

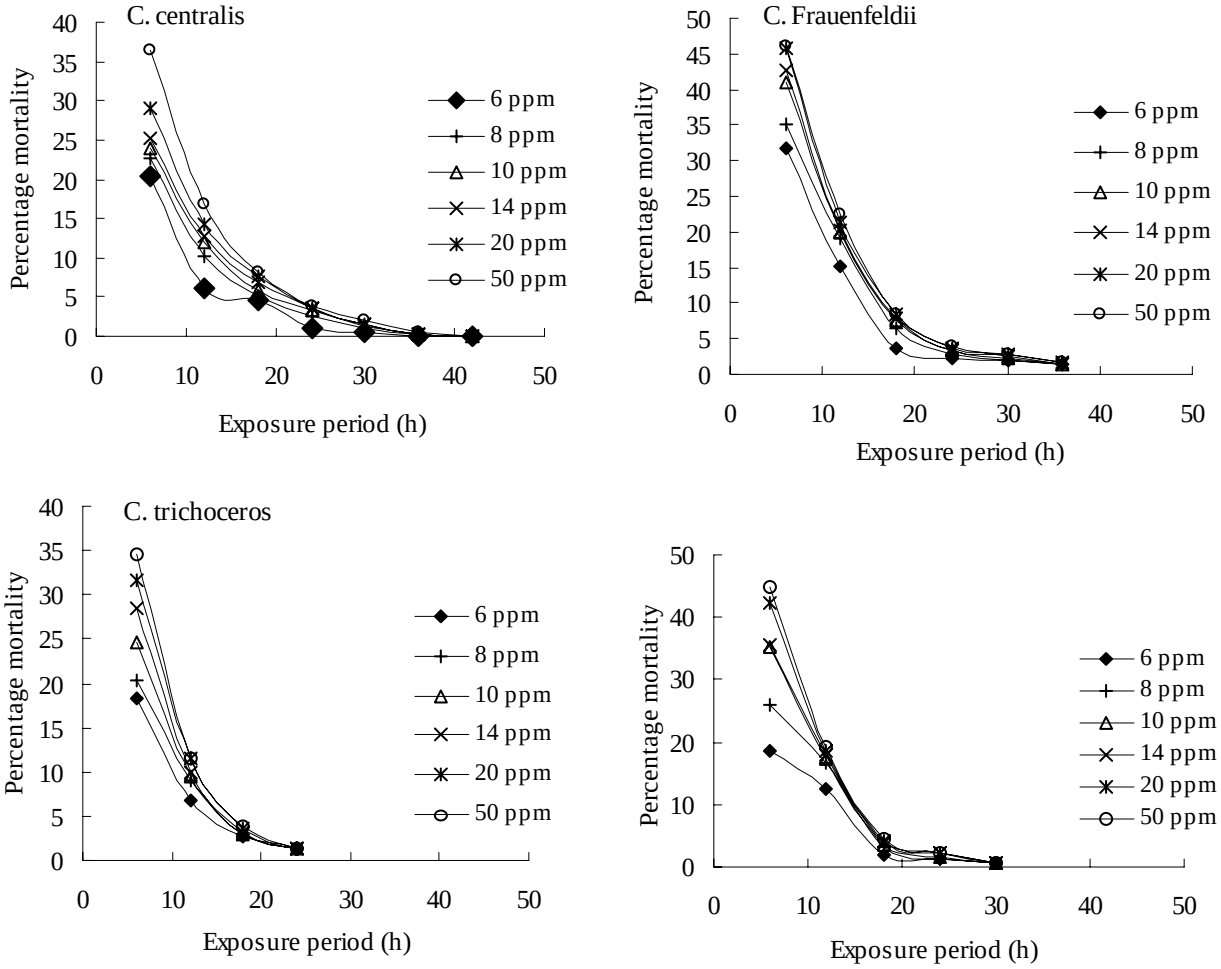

Fig. 5. Percentage toxicity of crude oil on population of C.centralis, C.trchoceros, T.frauenfeldii and O.mobiliensis as a function of at exposure period

demonstrating that crude oil input into the coastal waters is a risk factor to ecological status. A supporting evidence of crude oil toxicity to biota is found in the works of Obire and Anyanwu, (2009) which reported that in soils contaminated with concentrations of crude oil from 1 to 5%, higher concentrations of crude oil had adverse effect on fungal diversity.

As with most aquatic organisms, environmental stress due to the presence of xenobiotic pollutant in the habitat leads to altered physiological characteristics (in response to stress) and a greater degree of susceptibility to infections, disease and even death (Ruggiu *et al.*, 1998) so it probably was with the phytoplankton species in this study which resulted in mortality, though not 100%. The rate or extent to adjust to the crude oil induced-stress differed with the organisms. Results revealed that some phytoplankton species adapted more easily than the others. In this regard, *C.trichoceros* (dinoflagellate) appeared to resist crude oil stress more than the other three (diatoms). This was attributed to the fact that diatoms (*T.frauenfeldii*, *C.centralis* and *O.mobiliensis*) have lower reproduction and assimilation rates in comparison to dinoflagellate *C.trichoceros* characterized by rapid sexual or asexual reproduction (Klepel, 1992).

The general reduction of phytoplankton population by the added crude oil was attributed to factors such as inhibition of food consumption, decrease in cell size, cell number and bio-volume (Miller *et al.*, 1978; Klepel, 1992). Others were inhibition of photosynthesis, primary production and respiration mechanisms owing to the presence of hydrocarbons (Gordon and Prouse, 1973; Dunsan *et al.*, 1975; Miller *et al.*, 1978; Karydis, 1979; Kusk, 1980; Karydis and Fogg, 1980; Karydis,

1981; Dahl *et al.*, 1983). In a short term bioassay experiment (Miller *et al.*, 1978), oil in water showed photosynthetic inhibition at 5 mg/L crude oil within 24 hours and concentrations as low as 2 mg/L caused up to 50% reduction in photosynthesis (Miller *et al.*, 1978; Dahl *et al.*, 1983). Crude oil consists of up to 80 to 90% hydrocarbons (both aromatic and paraffin fractions), which adversely affect biochemical factors such as protein, and sugar contents and photosynthetic pigments (chlorophyll a and b) of phytoplankton cells (Miller *et al.*, 1978; Karydis and Fogg, 1980).The adverse effect of habitant change on three of the species could be closely related to the transition between the marine environment enriched with nutrients and the controlled systems with limited nutrient supply (Burridge and Shir, 1995; Barron *et al.*, 2002; Folder and Burns, 2003; Chindah, 2004; Carayo *et al.*, 2005; Cermeno, 2008; Nubi *et al.*, 2008). Dinoflagellate *C.trichoceros* which was the most affected could be linked to the fact that it is heterotrophic, very motile and active thus required more food for survival in comparison to diatoms (*T.frauenfeldii*, *C.centralis* and *O.mobiliensis*) which are unicellular and less motile (Miller *et al.*, 1978; Klepel, 1992). Opute (1990) added that the majority of dinoflagellates as well as diatoms found in Nigerian coastal waters manifest stenohaline peculiarities as they could not tolerate a wide salinity range, indicating sensitivity to habitat changes which might explain the natural mortalities observed for the species outside of sea water environment.

The inverse relationship between phytoplankton mortality due to crude oil and exposure period suggest that the negative effect of single dose crude oil in this concentration range may be temporary. This is in concordance with the works of (Miller *et al.*, 1978) in which further prolonged exposure did not evoke further harm of crude oil to phytoplankton species in a bioassay experiment. The observation might be associated with the characteristics of Nigerian crude oil reported to be light (moderately viscous) and characterized with relatively high evaporation loss (Eyong *et al.*, 2004; Igwebuike *et al.*, 2007). Greater adverse impacts with time could likely emanate from chronic oil inputs into the environment.Reports from literature (Kusk, 1980; Karydis and Fogg, 1980; Burridge and Shir, 1995) showed that at non-toxic levels of crude oil, phytoplankton metabolism could be enhanced due to increased nutrient availability from oil degradation, simulation of nitrogen-fixing algae and bacteria or increased leaching of nutrients from vascular macrophyte stands, reporting that concentrations less or equal to 100 ìg/L may stimulate growth but at up to 1.0 mg/L, growth in some species may be inhibited. The frequent occurrence of oil spills in the Niger Delta region of the country owing to afore mentioned factors undermine the chances of the presence of crude oil at non-toxic levels in this area (Awobajo, 1981; Ekekwe, 1981; Ibiebele, 1986).

CONCLUSION

Crude oil concentration in the range of 6.0 to 50mg/L evoked toxicity on four Nigerian indigenous phytoplankton species. In summary, resilience to habitat change from marine environment to laboratory conditions decreased in the order of *C.centralis* > *T.frauenfeldii* > *O.mobiliensis* > *C. trichoceros* but in relation to crude oil toxicity within 24-h contact time, vulnerability decreased in the order *T.frauenfeldii* > *O.mobiliensis* > *C.centralis* > *T.trichoceros* for the four species. This is a potential danger to phytoplankton species biodiversity conservation. Further toxicological research on these and other species, effects mediated through food web interactions as well as a field scale study incorporating natural ecosystem factors are recommended.

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