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EFFECT OF WASTE ENGINE OIL ON PHYTOPLANKTON OF THE CALABAR RIVER ESTUARY, NIGERIA.

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ABSTRACT

The effect of waste lubrication oil on phytoplankton of the Calabar River Estuary has been examined under static conditions in the laboratory. Significant growth inhibitions and growth pattern alterations as well as susceptibility to bacterial attack were observed in the dominant diatom species- *Actinocyclus* and *Aulocadiscus* species during a 5 day exposure to concentrations of the oil ranging from 14.5 to 58 ppm. Oil treatment also resulted in growth enhancements in nanoflagellate population. Considering the importance of diatoms to organic carbon and oxygen supply in the Calabar River and associated Cross River Estuary, their destruction by oil and/succession by flagellates will result in negative consequences for fisheries and overall ecology of the system. The high toxicity of the waste oil even at low concentrations of total hydrocarbons calls for a re-appraisal of the permissible levels of total hydrocarbons in Nigerian inland waters currently put at 10 ppm.

Key Words: Waste lubricating oil, Phytoplankton, toxicity, Calabar River Estuary.

INTRODUCTION

Hydrocarbon pollution is a worldwide problem affecting the atmosphere, soil and water. The risk of oil pollution is, however, greater in oil producing areas where accidental spills commonly occur during exploration, drilling, production and transportation (Preston, 1988).

In Nigeria, most aquatic environments contain high levels of hydrocarbons arising from chronic inputs of oily effluents from crude oil production platforms and refineries. According to Aguiyi-Ironsi et al (1988) offshore and onshore oil spills amounted to approximately 1.7 million barrels between 1970 and 1975. In 1998, damage to Mobil Producing Oil Company's underwater pipeline released over 40,000 barels of crude oil into wide areas of the Nigerian Gulf of Guinea while another 2,000 barrels was similarly spilled in Jones Creek area of the Niger Delta by Shell Development Oil Company. Apart from spills and accidental discharges of oil into the environment, another potential source of large scale but mostly undocumented oil pollution in Nigeria arises from indiscriminate discharges of crankcase oil by motorists and auto-workshops. According to Neff et al. (1976) the major anthropogenic sources of marine petroleum are those associated with transportation and surface run-off from land, much of which occurs in the biologically productive estuaries and nearshore waters. Studies have shown that over 17% of the total petroleum hydrocarbons released into coastal waters annually is made up of lubricating oil wastes (Van Vleet and Quinn, 1978).

Once in the marine environment, hydrocarbon can become diluted and dispersed by natural systems and eventually disappear through microbial action, oxidation, evaporation and deposition in sediments (Doerffer, 1992). According to GESAMP (1993) these processes and actions are slow and depending on location, character and concentration, hydrocarbons can be quite destructive to marine ecosystems. They can directly poison marine life, disrupt ecosystems by destroying juvenile forms of life and other links in the food chain and interfere with the communication and information gathering systems of animals (Smith, 1977).

The production and consumption of lubricating oils have been reviewed (Vazquez-Duhalt, 1989). The world production of lubricating oils is almost 1-2% of refined crude oil and world consumption is 35-40 million tones yearly. The world production of used motor oil is estimated at 25-28 million tones in addition to 12-15 million tones lost yearly during engine operations. In Europe the annual consumption of crankcase oil per car was 21.2 kg in 1975 and 12.5 kg in 1985. While new technology has helped to bring down the consumption in developed countries, in Africa, the consumption is expected to increase because of the large proportion of old and poorly maintained automobiles. Discharge of waste oil to the environment is also expected to be higher in Nigeria because unlike in developed countries where waste oil is re-used as fuel, incorporated in asphalt for roads and re-refined; it is largely discarded.

Used engine oil contains high levels of combustion-derived polycyclic aromatic hydrocarbons (PAH) and heavy metals such as lead (Table 1). It is considered as increasingly becoming a contaminant of concern because of the relatively large volumes entering the aquatic environment through sewers, urban run-off, highway run-off and other sources. A major cause for concern stems from linkages between combustion PAHs and carcinogenesis for which there is presently some circumstantial evidence in fish in highly polluted urban-associated waters (GESAMP, 1993).

Studies on the effect of petroleum hydrocarbons on marine biota are replete in literature (Doerffer, 1992). The effects of waste oil on survival and reproduction of the American flagfish have been studied in detail (Hedtke and Puglisi, 1980). Egg production was impaired by exposure to 3380 µl/l of the water-soluble fraction. The 30-day LC_{50} for larvae exposed as embryos was greater than 8100 µl/l and that for larvae unexposed as embryos was 755 µl/l. The maximum acceptable concentration of toxicant for water-soluble fraction of used crankcase oil was estimated to fall between 325 and 390 µl/l (approx. 0.1%).

Water extracts of No. 2 fuel oil and used crankcase oil were examined for their effects on algal communities in

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experiments lasting several weeks under nearly natural conditions (Bott and Rogenmuser, 1978). Exposure depressed chlorophyll concentrations (biomass), but microscopic observation and pigment ratios indicated that changes in dominant algal types were less dramatic than those exposed to water extracts of No. 2 fuel oil. Bate and Crafford (1985) studied photosynthetic rates in five phytoplankton species treated with water soluble extracts of used lubricating oil. Evolution of oxygen and assimilation of carbon were inhibited by relatively high concentrations of aromatics determined in reaction vessels.

Although the dominant lubricating oils entering the aquatic environment are used crankcase and engine oil, a number of industries, and especially the metal-working and transport industries, use a variety of cutting, lubricating, hydraulic and other industrial oils which can be expected to enter the environment at varying levels (GESAMP, 1993). GESAMP (1993) has given components of typical used automotive oils with respect to physical properties and concentrations of metals and organics (Table 1). The composition is expected to vary depending on levels of additives, state of machine and duration of use before discarding. In Nigerian cities, waste oils are dumped indiscriminately in the vicinity of automotive workshops, lubrication garages and drainage gutters etc., where they are eventually washed off by surface run-off into associated rivers.

The Calabar River Estuary is a dominant feature in the landscape of South Eastern Nigeria. The City of Calabar and associated developments, including the Export Processing Zone are situated on the East Coast and constitute important sources of contaminants into the river. The Calabar River Estuary is the largest tributary of the Cross River Estuary, which is the largest estuary in the Gulf of Guinea besides the Niger. According to Akpan (1994) the Calabar River Estuary is the largest producer of primary organic carbon in the Cross River system. Phytoplankton is dominated by the diatoms, *Actinocyclus* and *Aulocadiscus* species which serve as major food components to a wide range of commercially important fish species including the bonga-*Ethmalosa fimbriata* (Nawa, 1982, Akpan, 1997). Presently, there are no waste treatment facilities in Calabar. The Calabar River Estuary, therefore, serves as a major sink for municipal wastes especially during torrential downpours characteristic of the wet seasons. The last one-year has witnessed an unprecedented increase in vehicular traffic in the City. This has occurred as a result of the opening of the Calabar Port in the Export Processing Zone for import of fairly uses vehicles. Because most of the vehicles are old, with ages ranging from 10 to 15 years, their consumption and discharge of lubricating oils is expected to be on the increase.

The present study seeks to examine the impact that potential chronic contamination of the river by waste oil will have on the major primary producers and thus, on fisheries and overall ecology.

METHODOLOGY

Collection and acclimation of organisms

Phytoplankton were collected from surface waters of the Calabar River Estuary at Calabar Cement Company (CALCEMCO) jetty (Fig. 1). About 100 litres of the sample was filtered through 120 µm and 30 µm mesh nitex plankton net arranged in series. The 120µm mesh fraction, which contained mainly large zooplankton, and detritus was discarded. The 30µm fraction was transferred into a clean 1 litre glass vessel containing 200ml of dilution water. The plankton sample was maintained in plankton chamber for 24 hours, under 12 hour light-dark regime at approximately 500 lux intensity.

Preparation of dilution water

The dilution water was prepared from habitat water collected from the Calabar River Estuary as stated above but filtered through 0.45µm membrane filter under vacuum. The dilution water was analyzed for dissolved oxygen, pH, conductivity, nitrate, phosphate and silicate. Dissolved oxygen was measured using SCHOTT 867 DO meter; pH was determined with WTW pH-90 meter; conductivity was measured with WTW LF-90 meter. The nutrients, nitrate, phosphate and silicate were measured spectrophotometrically according to Parsons et al. (1984).

Preparation of test oil solutions

Waste oils from several autoworkshops were collected and pooled together. Water-soluble fraction of the waste oil was obtained by vigorously shaking the waste oil with dilution water in seperatory funnel. After allowing for complete phase seperation (approx. 6 hours), the aqueous extract was drained off into a precleaned glass vessel to serve as the stock extract. The concentration of total hydrocarbons in the stock extract was determined spectrophotometrically using n-hexane as the solvent. Spectrophotometric readings were obtained at 370 nm, which marked the wavelength of maximum absorbance for the waste oil in n-hexane. Test concentrations were computed by reference to calibration constant, obtained from standard concentrations of waste oil in n-hexane according to the equation:

$$C(ppm) = \frac{A' \ B' \ v}{V}$$

A is the absorbance of n-hexane extract of the waste oil extract.

$$B = \frac{\text{å} \ {}^{C_i}\!/\!{}_{A_i}}{n}$$

B is the calibration constant obtained from the equation:

C_i is the concentration of each standard solution

A_i is the absorbance of each standard solution

n is the total number of standard solutions

v is the volume of hexane used in extraction of waste oil extract (ml)

V is the volume of waste oil extract extracted with hexane (ml)

Test oil solutions were obtained from stock waste oil extract by appropriate dilution with dilution water to give 14.5 ppm, 29.0 ppm, and 58 ppm of total hydrocarbon. The control consisted of pure dilution water with no oil addition.

Experimental design

At the end of 24-hour acclimation of the organisms, phytoplankton, nanoflagellate and bacteria analysis was performed using Zeis inverted plankton microscope. After swirling to mix, equal volumes of the sample were added to test flasks (500 ml Erlenmeyer flasks) containing 200 ml of the specified concentrations of waste oil extract. The test vessels were maintained under similar conditions as during

Table 1: Characterization of used automobile oil

Parameter	Measured value
Gravity API at 16°C	24.0
Viscosity (cm^2/s)	0.99
Pour point (°C)	37
Flash point (°C)	140
Heating value (kJ/kg)	38000
Bottom sediment and water volume (%)	11.0
Sulphur (wt %)	0.43
Ash (wt %)	1.01
Arsenic (ppm)	5
Barium (ppm)	48
Cadmium (ppm)	3
Calcium (ppm)	1,850
Chromium (ppm)	7
Copper (ppm)	177
Iron (ppm)	1,025
Lead (ppm)	240
Magnesium (ppm)	559
Phosphorus (ppm)	1,250
Silver (ppm)	1
Tin (ppm)	58
Zinc (ppm)	480
Chlorinated solvents (ppm)	
dichlorodifluoromethane	20
trichlorotrifluoroethane	160
1,1,1-trichloroethane	200
Trichloroethylene	100
tetrachloroethylene	105
Total chlorine (ppm)	1,600
Other organics (ppm)	
Benzene	20
Toluene	380
Xylene	550
benzo[a]anthracene	12
benzo[a]pyrene	10
Naphthalene	330
PCBs	5

Table 2: Physicochemical characteristics of dilution water

Parameters	Concentration
Dissolved oxygen (mg/l)	4.4
PH	6.44
Conductivity (uS/cm)	510
Nitrate (mg/l)	0.55
Phosphate (mg/l)	0.009
Silicate (mg/l)	0.204

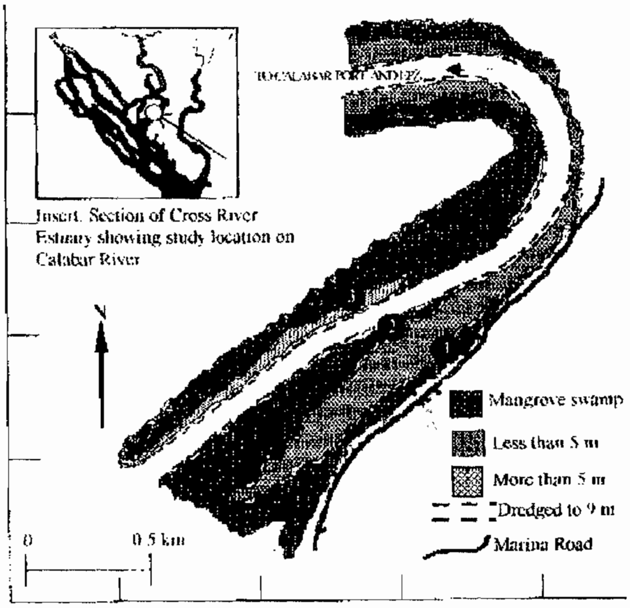

Figure 1: Map of study location showing sampling points on the Calabar River at CALCEMCO.

acclimation. Enumeration of organisms was carried out after 6 hours, 24 hours, 48 hours, 72 hours, 96 hours and 120 hours of exposure to oil.

Results

Results of physicochemical analysis of dilution water are given in table 2. Figure 2 shows the growth pattern of test organisms exposed to oil relative to controls. Waste oil produced marked changes in growth and growth patterns of the dominant diatoms, *Aulocadiscus* and *Actinocyclus* species relative to controls. After 120 hours (5 days) exposure to oil, both species were completely destroyed in contrast to the controls. Although high oil concentration (58 ppm) inhibited nanoflagellate growth, their growth was highly enhanced at medium concentrations of oil (14.5 to 29 ppm) relative to the controls. Bacteria attack also increased with increase in oil concentration.

Discussion

The marked suppression in growth of *Actinocyclus* species in response to waste oil contamination is of great significance to the fisheries and overall ecology of the Calabar River system. This is so because *Actinocyclus* is the most dominant and abundant diatom species in the estuary particularly during the dry season diatom bloom and contributes more than 90% of total density of phytoplankton during the period (Akpan, 1997). The allowable limit of total hydrocarbons in Nigerian inland waters is 10 ppm (DPR, 1991). As low as 14.5 ppm of waste oil extract inhibited *Actinocyclus* growth markedly (Fig. 2). Considering the indiscriminate practice of waste oil disposal in Calabar City and environs, this concentration can easily be attained and exceeded in the River particularly during periods of intensive input of materials through surface run-off. It is generally believed that diatoms contribute more oxygen to the atmosphere than do all the land plants combined (Wessels and Hopson, 1985).

In addition to the importance of *Actinocyclus* to the dissolved oxygen budget of the estuary, it is a major food for most commercially important fish species in the river including the bonga, *Ethmalosa fimbriata* and the Sciaenidae, *Pseudotolithus elongates* (Akpan, 1998). *Aulocadiscus* species is also a relatively abundant diatom second only to *Actinocyclus* in the Calabar River Estuary. According to Akpan (1998) the species is also capable of benthic existence and may actually be the most dominant form on the extensive mud flats and banks characteristic of the estuary. The marked alteration in growth curve of the species in response to oil additions is of great ecological significance in the Calabar River. According to Akpan (1998) the relatively high densities of phytoplankton in the highly turbid Calabar River Estuary particularly during the wet season may be attributed to input of benthic algae dominated by *Aulocadiscus*. Hydrocarbon related destruction of this source of primary organic carbon and dissolved oxygen input may result in marked destabilization of the ecosystem. Kausch (1990) noted that epipelic microphytes on mud flats and diatoms that migrate during low tides unto sediments surface are more important than phytoplankton for primary production in turbid estuaries since they can be exposed to full intensity of

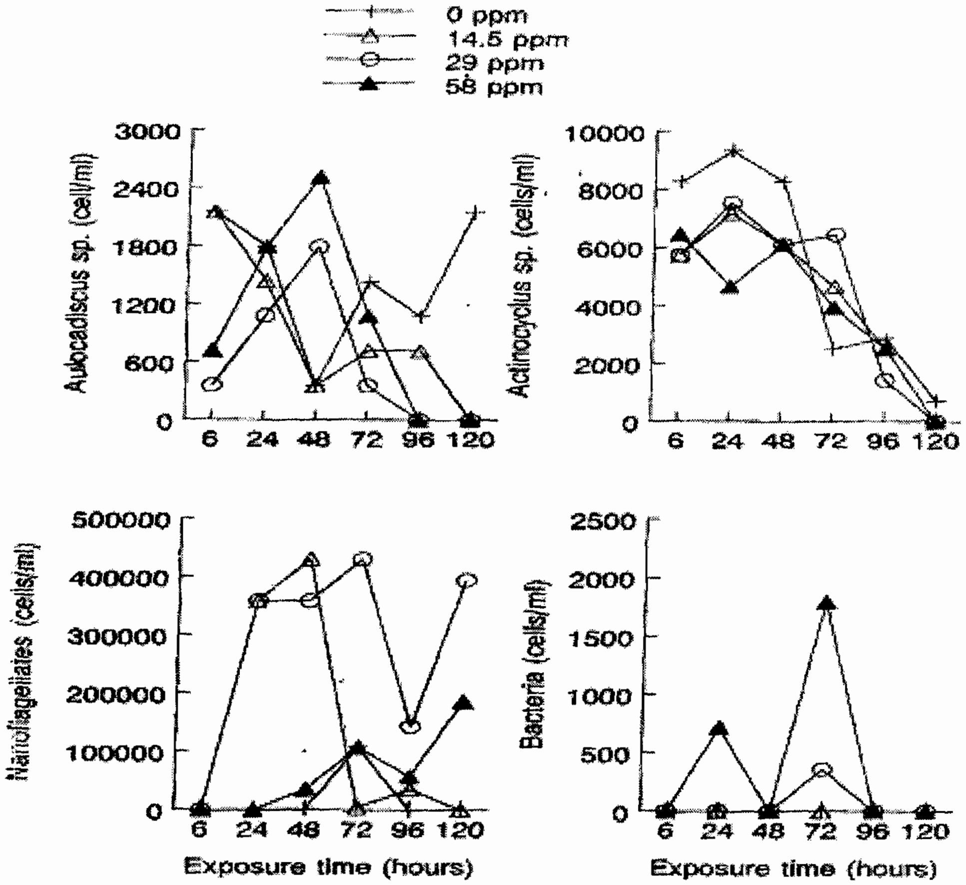

Fig. 2: Growth of phytoplankton and bacteria exposed to used-lubricating oil relative to controls

photosynthetically active radiation at least during some part of the daylight period.

Present results also revealed an explosion in growth of nanoflagellates in response to oil treatment with growth phase increasing with increase in oil concentration (Fig. 2). Although high oil concentration tended to inhibit flagellate growth initially, enhancement in growth was observed with increase in duration of exposure to oil treatment. According to Officer and Ryther (1980) flagellates, unlike diatoms, usually persist for longer periods of time, many are poor foods for most grazers, while many species are able, because of their motility, to concentrate to undesirable concentrations leading to serious pollution events including eutrophication and fish poisoning. During the 5th day of exposure when diatom growth was completely diminished by oil treatment, dense populations of flagellates remained, suggesting a succession of the highly desirable diatoms by nuisance flagellate species. The stimulation of flagellate growth by mineral hydrocarbons is a common phenomenon. In laboratory experiment with several species of marine phytoplankton exposed to a range of hydrocarbon concentrations, enhancement of photosynthesis was confirmed in the flagellate species *Chrysochromulina kappa* (Parsons et al., 1976). The effect was greater for aromatic hydrocarbons as opposed to straight chain alkane or alkenes. The authors also reported marked increases in density of natural community of phytoplankton subjected to No. 2 fuel oil under field conditions. The major species responsible for the increase was again found to be the haptophyte, *Chrysochromulina kappa*. Smith (1968) also reported a similar situation in the Torry Cayon oil spill in which a number of flagellates including *Chrysochrumulina*, appeared in the contaminated water.

Although growth enhancement in flagellate populations may be mainly attributed to the presence of growth regulatory compounds in oil (Table 1, Gordon and Prouse, 1973; O'Brien and Dixon, 1976; GESAMP, 1993), reduced competition due to destruction of dominant diatom populations by oil may be responsible for the initiation of the observed flagellate bloom. Limitations of phytoplankton growth and particularly, diatoms by mineral hydrocarbons is widely reported (Mironov, 1972, Lacaze, 1974, Vandermuelen and Ahern, 1976). Lubricating oil has been shown to have a greater potency of altering plankton growth compared to other mineral hydrocarbons (Gordon and Prouse, 1973, Nuzzi, 1973). Boylan and Tripp (1971) attributed the relatively high toxicity of the oil to the presence of high proportions of bi-cyclic and tri-cyclic aromatics in the water extracts of the oil. In addition to aromatic hydrocarbons, recent studies have shown that lubricating oils contain a wide range of heavy metals, which similarly have the potential to alter algal growth (GESAMP, 1993, Table 1).

Appart from direct impact of oil on diatoms, present results also revealed that oil treatment increased the susceptibility of diatoms to bacterial attack.

Ongoing planktological studies in the Calabar River Estuary has revealed other ecological roles played by the diatoms *Actinocyclus* and *Auclocadiscus*, in addition to primary production. They serve as substrates for the growth of the microcilliates, *Vorticella*, *Vaginicola* and *Epistilis* species. Although the nature of this symbiotic relationship is not yet clear, the growth of the ciliates on the diatoms certainly increases their nutritional value. Destruction of the diatoms by oil, will also lead to the destruction of their symbionts with serious consequences on the estuarine ecology.

In conclusion, waste oil as low as 14.5 ppm has been found to have the potential to destroy resident diatoms of the Calabar River Estuary and replace the diatoms with nuisance flagellates with negative consequences on the overall ecology, including eutrophication and fish poisoning. To avert the occurrence of this scenario, environmental laws regulating the use and disposal of mineral hydrocarbon products must be put in place. Strict penalties must be exerted on any offender. There is also the need for the Environmental Ministries and agencies to support and be involved in organized research towards acquisition of the necessary database for sound management of the environment

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