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Acute Toxicity Test of Oilfield Wastewater on Bacterial Community of a Soil in Nigeria

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ABSTRACT

Short-term effect of oilfield wastewater on the population and activity of soil heterotrophic bacteria was investigated from March to July, 2009. Soil samples (uncontaminated and wastewater-contaminated) were analyzed using standard procedures. Mean data of four stations for uncontaminated and wastewater-contaminated soils, respectively were CO_2 evolved: 36±2 and 37±1 mg 20 g^{-1}, Aerobic Heterotrophic Bacteria: 6.8±6 and 37±2×10^5 CFU g^{-1}, Anaerobic Heterotrophic Bacteria: 3.6±1 and 9.0±4×10^3 CFU g^{-1} and Total Hydrocarbon Content: 0±0 and 0.03±0 mg kg^{-1} soil. Values of microbial activity in terms of CO_2 evolution were approximately equal in uncontaminated and wastewater-contaminated soils which revealed no contamination effect. Also, addition of oilfield wastewater to the soil increased bacterial populations in the wastewater-contaminated soils above those of the control (uncontaminated) soils. Hence, the pollutant effect was positive rather than being depressive on the heterotrophic bacterial populations and no eco-toxicological effects occurred.

Key words: Short-term, effect, population, soil, activity, heterotrophic, bacteria, oilfield

INTRODUCTION

Toxicity is concerned with the effect of chemicals or toxic agents at the level of the individual organism or its constituent parts and emphasises the mechanistic bases of harmful effects and the conditions under which they occur (Klaassen and Eaton, 1991). It is a relative property of a chemical which refers to its potential to have deleterious effects on a living organism. Toxicity tests are used to evaluate the concentrations of the chemical and the duration of exposure required to produce the criterion effect.

Oil production activities release a large amount of hydrocarbon in terrestrial and aquatic environment. The level of soil pollution by petroleum products and oil sludge has approached millions of cubic meters (Zukauskaite and Viktorija, 2008; Sarma and Sarma, 2010). In addition, oil production activities discharge an aqueous solution referred to as oilfield wastewater. This water consists of "Formation water" and "Injected water". Formation water is the natural water that occurs in association with oil and gas deposits in reservoirs and being denser, it lies under the hydrocarbons (Wills, 2000). Injected water is additional water usually injected into the reservoirs to help force the oil to the surface in order to achieve maximum oil recovery. Both formation and injected waters are eventually produced as oilfield wastewater along with the hydrocarbons and

as an oilfield becomes depleted, the amount of oilfield wastewater increases as the reservoir fills with injected seawater (Wills, 2000). At the surface, oilfield wastewater is separated from the hydrocarbons, treated to remove as much oil as possible and then either discharged as oilfield wastewater into the sea or onto soil or injected back into the wells (Somerville *et al.*, 1987; Wills, 2000). Oilfield wastewater contains inorganic and organic constituents (Wardley-Smith, 1979); as well as hydrocarbon components (Koons *et al.*, 1977) and have variations in their chemical composition and behaviour.

Offshore oil production platforms as well as onshore oil fields discharge the oily water or wastewater into the environments as part of their normal operations (Somerville *et al.*, 1987; Obire and Wemedo, 1996; Wills, 2000). In offshore drilling for oil and gas, the wastewater is usually discharged into the aquatic environment whereas in onshore oil production it is discharged into wastewater stabilization ponds or onto open land as landfills. Soil contamination may also occur from accidental discharge of oilfield wastewater due to a crack which could develop in the wastewater stabilization ponds as a result of faulty construction or deterioration or absence of synthetic impermeable liners (Ream, 1983). Oilfield wastewater discharged onto terrestrial and/or into aquatic environment could be very devastating because this could lead to ecotoxicological and agro soil fertility problems that would create an artificial food scarcity due to damage to vegetation and aquatic organisms (Odeigah *et al.*, 1997).

The objective of this study, therefore, was to investigate the short-term effect of freshly collected oilfield wastewater on the population and activity of soil heterotrophic bacteria and to ascertain the ecotoxicological problems that could arise as a result of discharge of the wastewater into soil environment.

MATERIALS AND METHODS

Study area: The study area was a plain land around an oilfield location owned by Total Fina-Elf Nig. Plc. located in Elele-Okiniali community in Ikwerre Local Government Area of Rivers State, Nigeria. The land is made up of tropical rain forest vegetation in the Niger Delta area of Southern Nigeria. The oilfield location comprises of several oil wells and a flow station which is the central operational and controlling unit of the oilfield. The oil wells and flow station are spread and located within fallow farmlands used for agricultural purposes by the inhabitants of the area. Hence, the choice of the area was based on the fact that the area witnesses heavy and extensive farming activities yearly despite the petroleum operation activities. The oilfield is designated as "Olo" oilfield location divided into Olo 1(comprising of oil wells) and Olo 2 (consisting of oil wells and a flow station) oilfields about 300 m apart.

For the purpose of this study, four sampling stations, designated stations 1-4, were chosen and equally distributed between Olo 1 (Station 1 and 2) and Olo 2 (Station 3 and 4). Each station of the Olo 1 and Olo 2 oilfields has an area of 50 m^2 with a distance of 50 m apart. Station 1 is closest to the flow station about 20 m away.

Sampling: Sampling was carried out monthly for a period of five months and lasted from March to July, 2009. Oilfield wastewater and soil samples were collected from the study area. The wastewater samples were collected freshly-treated before it made contact with the environment and used in this form in the treatment of soil samples for toxicity test within two hours of collection. Freshly-treated oilfield wastewater samples were aseptically collected from the outlet of the separation/treatment plant of the oilfield flow station using sterile sample bottles. Prior to collection

of the wastewater samples, the interior of the nozzle of the outlet valve was flushed by allowing the water to flow to waste for 2 to 3 min this was done to avoid external contamination. After which the sample bottles were filled from a gentle stream of the wastewater.

Soil samples were randomly collected from the fallow agricultural lands of the four sampling stations. Surface soil (0-15 cm depth) was collected using a clean auger borer. To obtain the samples, the auger borer was dug into the soil down to the required depth (0-15 cm) and the bulked soil samples were put into fresh unused black polythene bags capable of holding the required soil quantity of 3 kg weight. Duplicated soil samples were obtained from each sampling station appropriately labeled and immediately transported to the green house of Department of Applied and Environmental Biology, Rivers State University of Science and Technology, Port Harcourt, for treatment.

Treatment of soil with oilfield wastewater: Duplicated soil samples of each sampling station (3 kg quantity) were placed into fresh unused polythene bags and properly labeled with the sampling stations. The sample bags were perforated at the base to allow excess water drain out. A set of the duplicate soil samples was treated by adding 500 mL of freshly collected oilfield wastewater and taken as wastewater-contaminated soil samples. Similarly, a second set of the duplicate soil samples was treated by adding a corresponding volume of sterile distilled water to serve as control. All the treated soil samples were allowed to stand for about 2 weeks (short-term incubation) at temperature of 32°C before taking samples for microbiological analysis. The treatments were repeated at 3 days intervals during the 2 weeks incubation period.

Determination of microbial activity: CO_2 evolution: For determination of carbon dioxide evolved from soils (uncontaminated and wastewater-contaminated), the methods of Flowers *et al.* (1984) and Cornfield (1962) were used and adapted using stoichiometric calculations. Twenty grams (20.0 g) of the soil samples were put into 500 cm^3 screw cap glass bottles. A vial containing 20.0 cm^3, 1 M NaOH was carefully placed in the bottle containing the soil samples and the bottle was quickly covered and tightly screwed. The bottles were incubated at room temperature for 7 days. Carbon dioxide evolved by the soil microorganisms reacted with the NaOH forming Na_2CO_3 and was measured titrimetrically. At the end of the incubation period, 10 cm^3 1 M $BaCl_2$ was added to the NaOH-CO_2 (i.e., Na_2CO_3) solution to precipitate the carbonate present. The precipitate was filtered out with Whatman No.1 filter paper. Then excess alkali in a known quantity (100 cm^3 used) of the filtrate was titrated with 1 M HCl titrant after 2 drops of methyl red indicator was added. This was to determine the amount of NaOH that did not react with the CO_2 evolved (to form carbonate) and was still present as OH in the solution. The time between opening the 500 cm^3 incubation bottles and titration was kept as short as possible to avoid much CO_2 from being absorbed from the atmosphere. This is because NaOH has much affinity for CO_2. For determination of the amount of carbon dioxide evolved per 20 g of soil sample used, the general titration procedure, followed by stoichiometric computations was used. Appropriate blanks were provided alongside the experiments to allow correction to be made for absorption of carbon dioxide from sources other than microbial respiration in the soil.

Enumeration of bacteria: Enumeration of bacteria from soil samples were performed using the ten-fold serial dilution method of Harrigan and McCane (1990) and Ofunne (1999). In this method, decimal dilutions of the samples were made by adding 1.0 g of soil samples to 9.0 mL of sterile normal saline (diluents) to give an initial dilution of 1:10 (10^{-1}). Subsequent serial ten-fold

dilutions were made by transferring 1.0 mL of the last dilution to 9.0 mL of fresh diluents up to 1:1000 (10^{-3}). Finally, 0.1 mL (aliquots) of the appropriate dilutions was plated out in duplicate onto the surface of suitable fresh dry sterile nutrient agar media in Petri dishes. The inoculum was evenly spread out with a sterile bent glass rod and incubated at temperature of 30±2°C for 24-48 h after which plates that had significant growth were counted and recorded. Anaerobic heterotrophic bacteria were placed in anaerobic jar containing liquid nitrogen in a small beaker and incubated under same condition as mentioned above.

Measurement of total hydrocarbon content in soils: Toluene extraction method of Odu *et al.* (1989) was employed. Five gram of the soil sample was measured into a beaker and 10 mL of toluene (Analar grade) was added to it. After shaking vigorously for 5 min, it was allowed to stand for 20 min. After which, two layers were formed and the supernatant (toluene-residual oil extract) was put into fresh test tubes (curvette). The hydrocarbon content (oil) extracted was determined spectrophotometrically at 420 nm using spectronic-20 Spectrophotometer. The absorbance reading was recorded after reading from a standard curve of the absorbance of different known concentrations of hydrocarbon extractant (toluene). Hydrocarbon concentrations were calculated by multiplying with the appropriate dilution factor and the results expressed as milligrams per kilogram (mg kg^{-1}).

Data analysis: Statistical analysis was carried out on the data to test for significant difference between the months, stations and soil-contamination status. Analysis of variance and Duncan Multiple Range Test were used to compare the data for the different months, stations and soil status. This was done using a Computer-based programme in Excel.

RESULTS

Data for microbial carbon dioxide evolution (mean of 5 months) of the soils at the sampling stations are presented in Table 1. Monthly data in all the samples analyzed ranged from 9.9-83.6, 20 and 6.6-92.0 mg 20 g^{-1} for control and wastewater-contaminated soils, respectively. Mean values of all the stations ranged from 15.6-58.7 mg 20 g^{-1} for control soils and 19.3-50.3 mg 20 g^{-1} for wastewater-contaminated soils and were 35.8 mg 20 g^{-1} for control soil and 36.8 mg 20 g^{-1} for wastewater-contaminated soils.

Densities of aerobic heterotrophic bacteria (mean of 5 months) of the soils at the sampling stations are shown in Table 2. Monthly aerobic heterotrophic bacterial counts (×10^5 CFU g^{-1}) in all

Table 1: Monthly, station values of microbial CO_2 evolution (mg 20 g^{-1}) of uncontaminated and oilfield wastewater contaminated soils

	Station							
	STN 1		STN 2		STN 3		STN 4	
Month	Control	Contam	Control	Contam	Control 1	Contam	Control	Contam
March	19.8	22.0	28.0	33.0	26.4	46.2	19.8	6.6
April	14.9	49.5	47.3	62.5	21.6	29.7	15.4	18.7
May	9.9	77.0	66.0	92.0	13.2	30.8	11.0	30.8
June	41.3	47.9	72.6	52.1	48.4	32.5	14.3	25.9
July	72.6	18.7	79.2	12.1	83.6	34.1	17.6	20.9
Mean	31.7	43.0	58.7	50.3	38.6	34.7	15.6	19.3

Contam: Contaminated, STN: Station

Table 2: Monthly, station counts of aerobic heterotrophic bacteria ($\times 10^5$ CFU g^{-1}) of uncontaminated and oilfield wastewater contaminated soils

	Station							
	STN 1		STN 2		STN 3		STN 4	
Month	Control	Contam	Control	Contam	Control	Contam	Control	Contam
March	20.1	34.0	17.9	38.0	9.2	32.0	9.8	20.2
April	19.1	29.3	3.3	63.0	1.45	33.0	3.20	53.0
May	05.50	28.8	6.0	44.0	1.35	30.2	2.05	23.6
June	04.80	37.0	4.2	9.6	12.60	12.2	4.60	29.9
July	01.95	64.0	4.2	49.0	1.85	51.0	1.95	52.0
Mean	10.30	38.0	7.1	41.0	5.30	32.0	4.30	36.0

Contam: Contaminated, STN: Station

Table 3: Monthly, station counts of anaerobic heterotrophic bacteria ($\times 10^3$ CFU g^{-1}) of uncontaminated and oilfield wastewater contaminated soils

	Station							
	STN 1		STN 2		STN 3		STN 4	
Month	Control	Contam.	Control	Contam.	Control	Contam.	Control	Contam.
March	5.5	5.3	3.6	9.6	2.91	13.0	4.1	15.4
April	4.6	2.36	2.75	4.1	2.17	10.5	3.3	11.5
May	5.3	5.7	2.7	8.8	2.37	10.4	4.1	15.8
June	4.6	5.9	2.65	9.4	2.32	10.4	4.3	16.5
July	4.3	5.3	2.2	7.8	2.71	10.4	5.1	01.63
Mean	4.8	4.9	2.8	7.9	2.50	10.9	4.2	12.2

Contam: Contaminated, STN: Station

Table 4: Monthly, station values of total hydrocarbon content (mg kg^{-1}) of uncontaminated and oilfield wastewater contaminated soils

	Station							
	STN 1		STN 2		STN 3		STN 4	
Month	Control	Contam	Control	Contam	Control	Contam	Control	Contam
March	0.00	0.01	0.00	0.00	0.00	0.02	0.00	0.00
April	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
May	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
June	0.00	0.37	0.00	0.00	0.00	0.00	0.00	0.00
July	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.03
Mean	0.00	0.08	0.00	0.00	0.00	0.01	0.00	0.01

Contam: Contaminated, STN: Station

the samples analyzed ranged from 1.35-20.1 for control soils; 9.6-64 for wastewater-contaminated soils. Mean counts of all the stations ranged from 4.3-10.3 and 32-41 for control and wastewater contaminated soils, respectively. Densities of anaerobic heterotrophic bacteria (mean of 5 months) of the soils at the sampling stations are shown in Table 3. Monthly counts of anaerobic heterotrophic bacteria ($\times 10^3$ CFU g^{-1}) in all the samples analyzed ranged from 2.17-5.5 for control soils and 1.63-16.5 for wastewater-contaminated soils. Mean counts of all the stations ranged from 4.3-10.3 for control soils and 32-41 for wastewater-contaminated soils.

Data for total hydrocarbon content (mean of 5 months) of the soils at the sampling stations are presented in Table 4. Values of total hydrocarbon content in all the samples analyzed were zero in all control soil samples and ranged from 0- 0.37 mg kg^{-1} in wastewater-contaminated soil samples. Mean values of the stations in control soils were also zero and in wastewater-contaminated soils it ranged from 0-0.08 mg kg^{-1}.

DISCUSSION

Results of microbial carbon dioxide evolution revealed high microbial activities in the soil samples except in a few samples where the activities were either low or moderate. Monthly values observed at the sampling stations were higher in control samples than in wastewater-contaminated samples in some months and reversed in the other months. Stations 1 and 4 recorded higher mean CO_2 values in contaminated soils than in control soils where the values were lower. But the reverse was the case in stations 2 and 3 where the control soil samples recorded higher mean CO_2 values and the contaminated soils recorded lower mean values. Station 4 recorded the lowest mean values in both soil status and station 2 recorded highest values. The mean data observed between the control and contaminated soils showed that contamination of soil with oilfield wastewater increased microbial activity in two stations and decreased activity in the other stations. However, mean of the four stations were slightly higher in contaminated soils than in uncontaminated (control) soils but approximately equal in both soil status, suggesting that the addition of oilfield wastewater to the soils did not alter microbial activities. There is no significant difference ($p<0.05$) between the months, stations and soil status.

Densities of aerobic heterotrophic bacteria in the soils during this study were generally high. Results of the monthly counts of aerobic heterotrophic bacteria at the sampling stations revealed that the contaminated soil samples had far higher microbial populations than the control soil samples. The monthly mean counts followed the same trend of variations between control and contaminated soils. The station differences in the mean counts of aerobic heterotrophic bacteria were noted. The data showed that station 4 recorded the lowest mean counts and station 1 had the highest mean counts for control samples whereas for contaminated samples, station 3 had the lowest mean counts and station 2 recorded the highest mean counts. The addition of oilfield wastewater to the soils increased the numbers of aerobic heterotrophic bacteria. There is significant difference ($p<0.05$) in the aerobic heterotrophic bacterial counts between the soil status and no significant difference between the monthly and station counts of the aerobic heterotrophic bacteria.

Results obtained for anaerobic heterotrophic bacteria in this study showed that the counts were generally moderate. The counts varied between the months, and stations and were generally higher in contaminated soils than in the control soil samples except in the months of March and April at station 1; July at station 4 where the reverse was the case. However, the mean counts were generally higher in contaminated samples and lower in control samples. The mean counts for control samples were lowest at stations 3 and highest at station 1; contaminated soils had lowest counts at station 1 and highest counts at station 4. The results showed that counts of anaerobic heterotrophic bacteria increased in contaminated soils after addition of wastewater. There is no significant difference in the counts between the months and station but significant difference ($p<0.05$) exist between the soil status.

Total hydrocarbon content of uncontaminated (control) and oilfield wastewater-contaminated soils was analyzed during this study to ascertain the extent of retention of oil contained in the wastewater by the soils. Values of total hydrocarbon content were zero in all control soils. Contaminated soils recorded zero value in most of the samples except in station 1 of March, June

and July; station 3 of March and station 4 of July which recorded very low values (<1.0 mg kg^{-1}). Mean values were also zero at all stations in control soil samples and very low in contaminated soil samples except at stations 2 which had zero value. The absence of hydrocarbon in the control soil samples was an indication that the study area had not been contaminated by crude petroleum. In the contaminated soils, only traces of oil were detected in a few samples while no presence of crude petroleum was found in the other samples. The explanation may be that the concentrations of crude petroleum in the wastewater were not high enough as to accumulate in the soil; more so the soil microbial community may have utilized the oil-in-water content of the wastewater faster than they could accumulate. Statistically, there was no significant difference between the months, stations and soil status. Koons *et al.* (1977); Obire and Wemedo (1996) reported that oilfield wastewater contains hydrocarbon components, even after treatment to remove the oil content. However, the hydrocarbon content of the oilfield wastewater used in this study could not impact negatively on both the soil microbial population and activity of the study area.

Generally speaking, the mean bacterial populations obtained during this study revealed that the counts increased in contaminated soils after addition of oilfield wastewater to the soils. Hence, the addition of the wastewater to the soils positively influenced the growth of bacteria and in turn, increased the microbial populations. However, statistical analysis revealed significant difference ($p<0.05$) in the counts of aerobic and anaerobic heterotrophic bacteria between the control and contaminated soils. This explains the fact that the increase in bacterial population in the wastewater-contaminated soils above those of control soils is significantly different; hence a real positive pollutant effect does exist.

Clark and Patrick Jr. (1987) and Obire (1988) stated that microorganisms are useful in predicting the impact of a particular stress on the environment and often respond to the introduction of a pollutant through shift in their numbers. McKinley *et al.* (1982); Clark and Patrick Jr. (1987) further argued that for the assessment of the potential for adverse environmental effect of pollutants on living sources to be significant, it should be based on the population of a representative species and not an individual species. In the present investigation, therefore, the assessment for the effect of addition of oilfield wastewater was based on the populations of two important physiological groups of bacteria (aerobic and anaerobic heterotrophic bacteria) and on microbial activity (CO_2 evolution). Bacterial populations generally increased after addition of oilfield wastewater; the contaminated soils recorded higher bacterial populations than the control soils. This observation agreed with the findings of Obire and Wemedo (1996; 2002) who reported that bacteria responded positively to the addition of oilfield wastewater to a soil. On the other hand, addition of oilfield wastewater to soils had little or no effect on activity of bacteria in the soil of the study area.

Nutrient concentrations really become limiting in times of pollution during which additional nutrients such as nitrate-nitrogen would be needed to enhance metabolic degradation (Atlas, 1981). In the case of oilfield wastewater in this study, the available oil and the inorganic constituents could have added to the carbon source and nutrient need of the soil respectively to support the growth and proliferation of bacteria in contaminated soils above that of uncontaminated (control) soils.

In conclusion, counts of bacteria were generally higher in contaminated soils than in control soils. The study revealed that addition of the oilfield wastewater to the soil increased bacterial populations of the oilfield wastewater-contaminated soils above those of the control soils. Hence, the pollutant effect was positive rather than being depressive on the heterotrophic bacterial populations and no eco-toxicological effects occurred. The microbial activity in terms of CO_2 evolution was not altered as to reveal contamination effect. The high heterotrophic bacterial populations and the values of CO_2 evolved showed normal microbial growth and activity in the soils.

REFERENCES

Atlas, R.M., 1981. Microbial degradation of petroleum hydrocarbons: An environmental perspective. Microbiol. Rev., 45: 180-209.

Clark, J.R. and J.M. Patrick Jr., 1987. Toxicity of sediment-incorporated drilling fluids. Mar. Pollut. Bull., 18: 600-603.

Cornfield, A.H., 1962. A sample technique for determining mineralization of carbon during incubation of soil treated with organic materials. Plant Soil, 14: 90-93.

Flowers, T.H., I.D. Pulford and H.J. Duncan, 1984. Studies on the breakdown of oil in soil. Environ. Pollut. Ser. B: Chem. Phys., 8: 71-82.

Harrigan, W.F. and M.E. McCane, 1990. Laboratory Methods in Food and Dairy Microbiology. 8th Edn., Academic Press, London, UK., pp: 136-138.

Klaassen, C.D. and D.L. Eaton, 1991. Principle of Toxicology. In: Casarelt and Doull`s Toxicology: The Basic Science of Poisons, Amdur, M.O., J. Doull and C.D. Klaassen (Eds.). 4th Edn., Pergamom Press, New York, pp: 12-49.

Koons, C.B., C.D. Mcauliffe and F.T. Weiss, 1977. Environmental aspects of produced waters from oil and gas extraction operations in offshore and coastal waters. J. Petroleum Technol., 29: 723-729.

McKinley, V.C., T.W. Federle and J.R. Vestal, 1982. Effects of petroleum hydrocarbons on plant litter microbiota in an Arctic Lake. Applied Environ. Microbiol., 43: 129-135.

Obire, O. and S.A. Wemedo, 1996. The effect of oilfield wastewater on the microbial population of a soil in Nigeria. Biologia, 1: 77-85.

Obire, O. and S.A. Wemedo, 2002. Seasonal effect on the bacterial and fungal population of an oilfield wastewater-polluted soil in Nigeria. J. Applied Sci. Environ. Manage., 6: 17-21.

Obire, O., 1988. Studies on the biodegradation potential of some micro-organisms isolated from water systems of two petroleum producing areas in Nigeria. Nig. J. Bot., 1: 81-90.

Odeigah, P.G.C., O. Nuradeen and O.O. Amund, 1997. Genotoxicity of oilfield wastewater in Nigeria. Hereditas, 126: 161-167.

Odu, C.T.I., L.C. Nwaboshi, S.O. Fagade and P.E. Awani, 1989. Post impact study of SPDC's Nun river and delivering line spillage. Final Report, SPDC, Nigeria, pp: 95.

Ofunne, J.L., 1999. Bacteriological Examination of Clinical Specimens. 1st Edn., Achugo Publications, Owerri, Nigeria, ISBN: 978-34685-4-5, pp: 24-35.

Ream, K.H., 1983. Hazardous Waste Management. American Chemical Society, Washington, DC., USA., pp: 8-12.

Sarma, A. and H. Sarma, 2010. Enhanced biodegradation of oil products by some microbial isolate supplemented with heavy metals. Int. J. Bot., 6: 441-448

Somerville, H.J., D. Benneth, J.N. Davenport, M.S. Holt and A. Lynes *et al.*, 1987. Environmental effect of produced water from North Sea oil operations. Mar. Pollut. Bull., 18: 549-558.

Wardley-Smith, J., 1979. The Prevention of Oil Pollution. Graham and Trotman Ltd., London, pp: 69-75.

Wills, J., 2000. A survey of offshore oilfield drilling wastes and disposal techniques to reduce the ecological impact of Sea dumping: The effects of discharges of produced waters. Ekologicheaya Vahktaa Sakhalina (Sakhalina Environment Watch) 25th May, Sakhalina, London, pp: 1-5.

Zukauskaite, A. and J. Viktorija, 2008. Impact of heavy metals on the oil products biodegradation process. Waste Manage. Res., 26: 500-507.