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Ecotoxicological effects of discharge of Nigerian petroleum refinery oily sludge on biological sentinels

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Ecotoxicological effects of the discharge of Nigerian petroleum refinery oily sludge on biological sentinels were examined. The ecotoxicological effects examined included acute toxicity tests on *Nitrobacter* sp., fresh water shrimp (*Desmoscaris trispinosa)* and brackish water shrimp (*Palaemoneles africanus*) from the aquatic environment. It also covered chronic toxicity tests on microbial nitrogen transformation activity in soil and the growth of the terrestrial fauna, earthworm (*Apporectoda longa*) in pristine soils spiked with predetermined concentrations of the sludge. Analysis of the Nigerian petroleum refinery oily sludge used in this study indicated that the sludge is slightly acidic with a high total petroleum hydrocarbon (TPH) content of 340,000 mg/kg made up mainly between 10-40 carbon unit compounds. The sludge reduced the growth of *Nitrobacter* sp. in aqueous medium and also caused chronic effect on microbial nitrogen transformation activity in soil because it exceeded the 25% inhibition limit for chemicals with the potential to cause chronic effects on soil microbial activities. Similarly, the sludge exhibited toxicity on fresh and brackish shrimp. The freshwater shrimp was however more affected with an LC_{50} of 1097.375 ± 0.62 mg/kg when compared with an LC_{50} of 1590. 37±0.92 mg/kg obtained for the brackish water shrimp. It also reduced the growth rate of the earthworm (*A. longa)* progressively as the sludge concentration increased.

Key words: Toxic effects, petroleum refinery oily sludge, biological sentinels.

INTRODUCTION

Petroleum sludge are oily and viscous residues, which are formed during production, transportation, refining of petroleum and storage. Petroleum refinery oily sludge is composed basically of oil, water and solids (Ururahy et al., 1998). The oil industry is responsible for the generation of high amounts of oily sludge as waste by-product. However, one of the problems faced by the oil industry is the safe disposal of the oily waste generated. It is estimated that approximately 1% of the total oil processed in a refinery is discarded as oily sludge (Ururahy et al., 1998). These oily wastes are expensive to store or destroy and previously contaminated areas have required expensive remediation processes to minimize contaminant dispersion.

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Improper disposal of petroleum refinery oily sludge leads to environmental pollution, particularly soil contamination, and poses a serious threat to ground water. Many of the constituents are carcinogenic and immunotoxicants (Prospt et al., 1999). The polycyclic aromatic hydrocarbons (PAHs) have also been known to impair chemoreceptor functions in aquatic lives and hence lead to extinction of some species. They have also been known to bioaccumulate up the food chain, resulting in cancers and other genetic malfunctioning in man and other higher animals (Atlas and Bartha, 1992).

Several disposal options for petroleum refinery oily sludge disposal include thermal treatment (incineration) (Ururahy et al., 1998), landfills (Mishra et al., 2001) and biotreatment using the following methods: composting, land farming and biopile (Englert et al., 1993). Other conventional biological treatment methods include activated sludge and anaerobic digestion (Hazardous Waste Management, 1997). An interesting alternative to circumvent these problems is the use of bioreactors since optimum process conditions can be easily controlled, allowing higher quality final effluent in shorter times. However, they might have high costs (Oolman et al., 1996).

The Department of Petroleum Resources (DPR), an agency under the Federal Ministry of Environment (FMENV), which regulates activities in both the upstream and downstream petroleum sector, recommends that petroleum refinery oily sludge should be treated and disposed by method that shall not endanger human life and living organisms and cause significant pollution to ground and surface water (DPR, 2002, part iii sec. D 3.6.31, 12). Such approved methods include recycling (resource recovery), incineration, solidification, land farming (bioremediation) and land filling.

Toxicity tests are used to expose test organisms (fish, shrimps, microorganism, earthworms) to a medium–water, sediment, or soil and evaluate the effect of contamination on the survival, growth, reproduction, behavior and or other attributes of these organisms. These tests may help to determine whether the contaminant concentrations in a site's media are high enough to cause adverse effects in organisms. Acute toxicity tests are short term tests that measure the effects of exposure to relatively high concentrations of chemical. Chronic toxicity test, on the other hand, generally are longer– term tests that measure the effects of exposure to relatively lower, less toxic concentrations. Toxicologists have based their selection of test organisms (sentinels) on several factors; sensitivity to variety of substances, availability, representativeness of a variety of ecosystems, and ease of maintenance and culture under laboratory conditions. Sentinels are biological indicators that can help define the ecotoxicological effects of environmental contaminants (USEPA, 2004). In view of the problems encountered in the management of petroleum refinery sludge, this study examined the acute and chronic ecotoxicological effects of Nigerian Petroleum refinery oily sludge on biological sentinels.

MATERIALS AND METHODS

Sample collection

The petroleum refinery oily sludge used for this study was collected from petroleum refinery oily sludge holding tank of Warri Refinery and Petrochemical Company Ltd (WRPC), Warri, Ekpan, Delta State, Nigeria in 2 L glass bottle and preserved at 4°C until required for use. WRPC lies within the coordinates 5° 32' 15''N 5° 41'41''E.

The *Nitrobacter* sp. bacteria used was isolated from Aladja River, Aladja (5° 14'4''N, 60 15' 18''E) Delta State, in southern Nigeria. DSMZ heterotrophic nitrobacter medium was used for the isolation of bacteria. Isolates that were grayish, mucoid, flat, Gram negative, pear shaped and aerobic were selected according to the scheme of Colwell and Zambrushki (1972). Subcultures were made into slants of DSMZ– nitrobacter agar and stored at 4°C until required for use.

The shrimps were collected from the wild at Abua and Bomadi in Rivers and Delta states of Nigeria, respectively. Coordinates for Bomadi, Delta State are 5° 10' 0 N, 5° 56' 0''E. Abua in Rivers State is 10b miles from Port Hacourt with coordinates 5° 18' 30"N, 6° 25' 0''E Transportation of the shrimp to the laboratory was in air-inflated bags with the organisms' habitat water. Physicochemical parameters of the water were determined using standard methods (APHA, 1998).

The earthworm (*Aporrectoda longa)* commonly found in southern Nigeria was collected from a farm at Ubogo, Delta Southern Nigeria. The worms were collected according to the method described by Terhivuo et al. (1994) and Spiegel (2002). They were collected by digging and hand sorting from subsurface litters and taken to the laboratory for identification. They were then washed free of adhering soil particles and left on moist filter paper for voiding. Earthworms were selected based on their maturity (shown by the presence of clitellum) and liveliness (active response when anterior segment is prodded). The physicochemical parameters of the native soil were determined prior to the test.

Acute toxicity of petroleum refinery oily sludge pollution on the bacteria (*Nitrobacter* sp.), brackish water and freshwater shrimps

A range finding test was first conducted using three concentrations after which a definitive test was conducted with five geometric concentrations based on the result of the range finding test. The methods of Duffus (1980), Wang (1984) and APHA (1998) were adapted with some modifications. A fresh dilution and culture was made from the *Nitrobacter* sp. slant. A loopful of the bacteria was collected from the slant and dislodged in 20 ml peptone water and allowed to stand for a few hours at 30°C. The stock culture was prepared by inoculating 180 ml of sterilized peptone water with 20 ml of the activated culture. The test concentrations were prepared using the dilution water from the bacteria's habitat, sterilized at 121°C for 15 min.

Concentrations of sludge used were 5000, 2500, 1250, 625 and 312.5 mg/l. One hundred milliliters (100 ml) of each test concentration was then put into a 250 ml conical flask and sterilized at 121°C for 15 min. On cooling, 10 ml of the cell suspension was added to each flask containing the different sludge concentration and control (sterile dilution water). This was done in triplicate. The flasks were shaken thoroughly to mix and were incubated at 30± 2°C to determine the number of viable cell at 0 (start), 8, 16 and 24 h. 0.1 ml of each test concentration and the control was collected from the test solution and dispersed onto the surface of an already prepared DSMZ nitrobacter agar plate. The plates were then incubated at 30°C for 24 h. The viable cells were counted and

recorded. The EC_{50} was determined using the probit method of analysis (Finney, 1978). The following parameters were determined on the test solutions; pH, conductivity, dissolved oxygen, TDS, ammonia, alkalinity and sulphide. The methods used were adapted from APHA (1998).

Acute toxicity of freshwater shrimp (*Desmoscaris trispinosa*) and brackish water shrimp (*Palaemonetes africanus*) exposed to petroleum refinery oily sludge was determined using the method recommended by OECD #218 (2004). The sediment used in the toxicity test was collected with the aid of hand held van Veen type grab. After the collection, the sediment was sieved through a 500 µm mesh using dilution water in order to remove any organisms, which may interfere with the test. The sediment was allowed to settle overnight and the supernatural water decanted. The sediment was then stored in the dark at 4°C until required for the experiment. Approximately 24 h prior to testing, the sediment samples were removed from the refrigerator storage and allowed to equilibrate to room temperature and weighed.

A preliminary range-finding test was conducted prior to the actual test to assist in determining the appropriate dilutions. The range finding test was conducted using a broad concentration range (100, 1000 and 1000 0mg/kg) and the test was terminated in 24 h. In the definitive test, the concentrations selected were based on the mortality values obtained from the range finding and were in appropriate logarithmic dilution series. The 10-day static sediment bioassay was conducted by placing the weighed sediment into triplicate sets of 5 L amber coloured glass tanks. Concentrations of 625, 1250, 2500, 5000 and 10000 mg/kg of sludge were prepared in three replicates and properly homogenized with the sediment and were spread evenly in each tank. The sediment was then overland with 2 L of water from the organism's habitats. The contents of the containers were left to settle for 2 to 3 ho prior to the addition of the test organisms. Shrimps were collected with a 500-µm mesh sieve and placed in dilution water to rinse off any debris. Ten (10) shrimps were gently transferred into each glass test-tank containing test chemical (sludge) and control. The overlaying water was gently aerated for the 10-day's exposure period. Observations for mortality in the test – vessels were made and records taken for the numbers of shrimp which were swimming, crawling on the surface, loss of appendages emergency of organisms from sediment, immobilized (lying on the sediment surface but obviously still alive) or dead. Dead shrimps were removed at each observation. After 10 days, the sediments were sieved and the number of dead shrimps recorded. Average mortality in the bioassay, that is, the total number of organism used on day 0 was used to estimate the average percentage mortality in the bioassay at day 10. The LC_{50} was also determined by the probit method (Finney, 1978). Controls with clean sediment (without sludge) were conducted along with the treated sludge (Environment Canada, 1992). The size of test shrimps used was 0.156±0.03 g and 2.65±0.36 cm in length for the freshwater and brackish water shrimp, respectively. The physicochemical parameters of the test solutions were determined 24 hourly for 96 h.

Chronic toxicity (sublethal) effects of petroleum refinery oily sludge pollution on nitrogen transformation activity in the soil

The OECD TG 216 (2000) test method was used for this test. The effect of the petroleum refinery oily sludge on the nitrifying bacteria, *Nitrobacter* sp. was determined. This test was used to detect long-term (chronic) adverse effects of petroleum refinery oily sludge to the process of nitrogen transformation activity in the soil. Pristine soil was taken from a depth of 0 to 20 cm from a garden in Orhuwhorun town in Delta state, Nigeria and was transported in an ice – chest at 4°C to guarantee the initial soil properties were not significantly altered. In the laboratory soil, samples were kept in the refrigerator at 4± 2°C when they could not be used immediately. The soil was dried, sieved and amended with 5 g/kg compost and treated with five concentrations (375, 6250, 12500, 25000 and 50,000 mg/kg) of petroleum refinery oily sludge or left untreated (control) after day 0, 7, 14 and 28, treated control samples were extracted and analyzed for ammonia, nitrate and *Nitrobacter* sp. counts, the rate of ammonia nitrate formation in treated soil was compared with rate in the controls and the percent deviation of the treated from control was calculated. Enumeration of nitrogen transformation bacteria (*Nitrobacter* sp.) was also done to correlate the microbial growth with the transformed nitrogen. Results from the test of multiple were analyzed using a regression model (ANOVA) and the EC_{50} was calculated. All analyses were done by ASTM method. The control contained only the soil. A geometric series of five concentrations was used. Three replicates for both treatments and control were used.

The test was carried out in the dark at 25± 20°C where 90 ml of sterile distilled water was added to each tank to achieve moisture content of between 40-60%, while 60 ml was added to the 50,000 mg/kg test – tanks to achieve the same percentage of moisture content. The content of the tank was mixed thoroughly and covered with perforated polythene to prevent excessive evaporation of water and volatile fractions. Moisture content of between 40-60% of the maximum water holding capacity was maintained during the test by watering at intervals with distilled water. The duration of the test was 28 days. Composite soil sampling was done on days 7 and 28 and the soils samples were analyzed for some physico-chemical parameters such as pH, total petroleum hydrocarbons, total organic carbon, polyaromatic hydrocarbon nitrate and ammonia. microbio-logical parameters included enumeration of total heterotrophic bacteria and *Nitrobacter* sp. count. Enumeration of *Nitrobacter* sp. was done with DSMS heterotrophic nitrobacter medium.

The quantity of ammonia and nitrate formed and *Nitrobacter* sp. counts obtained in each replication test soil were recorded, mean values of all replicates were determined and a dose response curve was prepared for the estimation of the effective concentration causing 50% reduction (EC_{50}value). The rate of nitrate formation in treated samples was compared with the rate in the controls, and percent deviation/inhibition of the treated from the control was calculated after 28 days using the formula below (Grunditz and Dalhammar, 2001):

$$\text{Inhibition (\%)} = \frac{C_{ref} - C_{sample}}{C_{ref}} \times 100$$

Where C_{ref} is concentration of nitrate formed in control, C_{sample}

Chronic toxicity effects of petroleum refinery oily sludge on the growth and survival of earthworm (*A. longa*)

Once organisms were obtained, they were identified and maintained in the laboratory using the procedures described in ASTM standard E2172 – 01 (ASTM, 2001). The selected worms were acclimatized for 1-7 days in the soil from the organisms' habitat. During this period, the worms were fed with cellulose. Cellulose was prepared in advance by shredding white, kaolin based paper, followed by converting it to pulp by mixing with distilled water, and subsequently drying at 30°C for 48 h. The weight of the worms was between 300 and 600 mg. Test conditions used were: temperature 20 ± 2°C, light-dark cycles; 16 and 8 h. The test earthworms used for this study were ecologically relevant to the Niger Delta of Nigeria. They possess the following characteristics, red – violet in colour, anterior black segment, a prolobous postonium, 8-14 cm in length, about 149 – 157 segments, a bar-shaped tubercular and alternately paired ternatogential tumescences.

Four concentrations (375, 750, 1500 and 3000 mg/kg) of the petroleum refinery oily sludge were prepared for the definitive test

after a preliminary range-finding test was conducted for two days. Five hundred grams (500 g) of soil were mixed with various test concentrations of the sludge and 20 g of cellulose. Cellulose was added to the soil as food for the earthworms. These were manually homogenized and distilled water (80 ml) was added to achieve 45% moisture content in one litre (I L) amber-coloured glass jars. A blank (control) containing only cellulose, water and soil was also prepared. Test tanks were prepared in triplicates per concentration. Prior to use for the test, chosen worm were stored for 24 h on a damp filter paper to void contents of the stomach and intestinal tract. The ten (10) earthworms selected were placed on the surface of the control and test soil samples and were allowed to ingest and burrow into the test medium. The distribution of individual earthworms among the test chambers was randomized. The test medium and control were analyzed for pH, TPH content, total organic carbon $(TOC)_g$ metals (chromium, cadmium, nickel, iron and copper), at the start of the experiment and weekly for 28 day.

Death was the primary criterion used in this test guideline to evaluate the toxicity of the test substance. Earthworm in the test and control chambers were observed weekly for 28 days and the number alive were recorded and the dead removed. In addition to death, weight loss, behavioural symptoms and pathological symptoms were recorded. Each test and control chambers were for dead or affected earthworms and observations recorded on day 7, 14, 21 and 28 days after the beginning of the test. Missing earthworms were considered dead. Mortality was assessed by empting the test medium on a glass or other inert surface and the earthworms were sorted from test mixture and their reactions were tested by a gentle mechanical stimulus. Any adverse effects (eg weight loss, behavioural or pathological symptoms) were noted and reported. The 28 day test result would be unacceptable if more than 20% of control organisms died or the total mean weight of the earthworm in the control containers declined significantly during the test (by 30%). The sublethal effects and growth (fresh weight) data were used to determine the EC50 using the Probit software. ANOVA was used to test for significant differences between treatment means and the control. At the end of the test, worms were removed from each jar, washed, dried, counted and weighed. Observations such as motility, light sensitivity and physical qualities (discolouration), morphology (open wounds) were documented to provide some indication of toxic response. The worms were then depurated for 24 h to void contents of the intestinal tract and subsequently rewashed and reweighed. The worms were analyzed for TPH and metals concentrations.

RESULTS

The physicochemical and microbial qualities of Nigerian petroleum refinery oily sludge shown in Table 1 indicates the sludge was acidic with a pH value of 5.81 ± 0.28 and total petroleum hydrocarbon (TPH) of 340000 ± 50000 mg/kg. Polyaromatic hydrocarbon (PAH) content of the sludge was 0.075 ± 0.02 mg/kg while the value for ammonium was 21.65 ± 1.21 mg/kg. The heterotrophic bacteria and fungi counts were 5.86E + 05 and 4.72E + 05 cfu/g, respectively. Hydrocarbon degrading bacteria and fungi counts were 2.85E + 02 and 2.75E + 02 cfu/g, respectively.

As shown in Table 2, the counts of *Nitrobacter* sp. after 24 h exposure to five concentrations of petroleum refinery oily sludge used dropped from 4.92E + 07 to 2.52E + 06 cfu/ml from the lowest concentration (312.50 mg/l) to the highest concentration (5000 mg/L) of the petroleum refinery oily sludge, respectively. *Nitrobacter* sp. counts obtained in the experimental control were 2.68E + 09 cfu/ml.

As shown in Table 3, the chronic toxicity effects of petroleum refinery oily sludge on nitrogen transformation activities in soils showed that the percentage inhibition of nitrogen transformation in petroleum refinery oily sludge contaminated soils in relation to the control increased with increasing sludge concentration. The increase ranged from 18.7 to 79.38% from the lowest concentration of 3750 mg/kg to the highest of 5000 mg/kg, respectively.

Since in our analysis of metal contents of sludge, zinc recorded a high concentration of 100.62 mg/kg in relation to other metals analyzed, it could have contributed to the observed inhibitory effect of the sludge on the organisms used in this study as observed in similar studies by Wang and Reed (1984). They noted that a high concentration of metal cations inhibits microbial activities by causing damage or inactivating one or more critical enzymes resulting in formation of an inactive complex between the metal cations and an active enzyme. TPH contains toxic compounds such as PAHs and these have also been implicated in the inhibition of nitrification process (Suschka et al., 1996; Dokaniakis et al., 2005)

Chronic toxicity profile of nitrogen transforming bacteria exposed to petroleum refinery oily sludge for 28 days recorded an EC_{50} of 13761.059 mg/kg as show in Table 4. This indicates that at the obtained EC_{50}, there would be 50% inhibition of nitrogen transformation activity in the soil. The recommended limit for non-chronic effect on nitrogen transformation activity in soil is ≤ 25% (ISO/DIs 14248; 1995). This would be a sludge concentration of 502.41 mg/kg. The sludge concentration recorded as the EC_{50} and above would result in serious inhibition of nitrogen transformation activity in soils and subsequently result in soil infertility.

The mean acute toxicity profile of the fresh and brackish water shrimp exposed to varying concentrations of petroleum refinery oily sludge in the sediment shown in Table 5 indicate that there were no death or physiological changes in the negative control for the 10-days test duration. The control shrimps appeared active and healthy (responsive to stimuli) throughout the test period. The test organisms exposed to the various sludge concentration had higher mean percentage mortality by day 10 in the fresh water test (40, 57, 67, 80 and 100%) than in the brackish water test (33, 50 63, 73 and 93%) in 625, 1250, 2500, 5000 and 10,000 mg/kg respectively (Figure 1). These results indicated that mortality increased with increased sludge concentrations and exposure duration. The toxicity profile indicated the estimated mean LC_{50} at day 10 for the fresh water shrimp was 1097.375 ± 0.620 mg/kg while that for brackish water shrimp was 1590.376 ± 0.920 mg/kg. GESAMP rating indicates both LC_{50} to be hazardous (Table 5).

Effect of petroleum refinery oily sludge on organism in the terrestrial environment was also determined with earthworm (*A. longa)* bioassay shown in Table 6.

Table 1. Physicochemical and Microbiological properties of Nigerian Petroleum refinery oily sludge.

Parameter	Mean (± S.E).
pH	5.81± 0.28
Conductivity, us/cm^2	466.65 ± 25.25
Sulphate, mg/kg	4.83 ± 0.64
Nitrate, mg/kg	26.40 ±1.02
Phosphate, mg/kg	7.73 ± 0.88
Total Nitrogen, mg/kg	0.12 ± 0.5
Total Petroleum Hydrocarbon, mg/kg	340,000 ± 50,000
Polyaromatic Hydrocarbon, mg/kg	0.075 ± 0.02
Hydrocarbon Degrading fungi, *cfu/g*	2.85 x 10^2
Ammonium, mg/ kg	21.65 ± 1.21
Copper, mg/kg	5.53 ±0.20
Chromium, mg/kg	8.68 ± 0.03
Nickel, mg/kg	3.36 ± 0.02
Cadmium, mg/kg	0.32 ± 0.5
Zinc, mg/kg	100.65 ± 2.30
Barium, mg/kg	0.31 ±0.04
Heterotrophic Bacteria, cfu/g	5.86E +05
Heterotrophic Fungi, cfu/g	4.72E+ 05
Hydrocarbon Degrading Bacteria, cfu/g	2.75E + 05

Table 2. Growth of *Nitrobacter Sp* Exposed to Petroleum refinery oily sludge after 24hrs

Exposure time (h)	Control Counts (CFU/ml)	Microbial Counts CFU/ml				
		Concentration of sludge (mg/l)				
		312.50	625	1250	2500	5000
0	2.88E +07	7.40E +05	5.10E + 05	3.60E + 05	2.40E + 05	2.20E + 05
	2.96E + 07	7.22E + 05	5.42E + 05	3.36E + 05	2.62E + 05	1.82E +05
8	6.78E + 08	5.36E + 06	4.88E + 06	2.88E + 06	6.88E + 05	3.96E + 05
	7.26E + 08	6.12E + 06	5.22E + 06	2.24E + 06	7.12E + 05	4.22E + 05
24	2.16E + 09	5.36E + 07	3.64E + 07	1.36E + 07	7.20E + 06	2.40E + 06
	2.68E + 09	4.92E +07	3.12E + 07	1.42E + 07	6.88E + 06	2.52E + 06

Table 3. Percentage (%) inhibition of Nitrate formation in Petroleum refinery oily sludge contaminated soil

Sludge concentration (mg/kg)	% inhibition
3750	18.66
5250	33.04
12500	51.70
25000	59.82
50000	79.38

Earthworms are associated with a healthy soil and their absence is an indication of poor soil health (Doube and Schmidt, 1997; Edwards and Shipitalo, 1998; Parmelee et al., 1998). This chronic effect studies of petroleum refinery oily sludge on earthworms showed that the sludge led to a reduction of growth progressively as the concentration of the sludge increased (Figure 2). The growth rate was inhibited from 86.71 (375 mg/kg) to 37.92 (3000 mg/kg). While 825.03 mg/kg was obtained as the EC_{50} at the end of 28 days (Table 6). This concentration reduced the growth rate of the test earthworms by 50%. In comparison with toxicity ratio of chemicals to earthworms (Davies, 2003), the EC_{50} value indicates the sludge as slightly toxic (Table 6). The values obtained as biocentration factor (BCF) for TPH concentration in the earthworm ranged from 1.22 to 5.17,

Table 4. Chronic Toxicity Profile of Nitrogen Transforming Bacteria Exposed to Petroleum Refinery Oily Sludge for 28 Days.

Days limit	EC_{50} (mg/kg)	Confidence	Probit equation	Slope
7	Could not be determined growth growth inhibition <50%	Could not be determined inhibition <50%	Could not be determined growth inhibition <50%	Could not be determined growth inhibition <50%
14	Could not be determined growth growth inhibition <50%	Could not be determined inhibition <50%	Could not be determined growth inhibition <50%	Could not be determined growthinhibition <50%
21	24627	19317-33858	-0.110 + 1.164X	7.82
28	13761	11796- 17418	-0.881+1.415x	5.005

Table 5. Acute toxicity profile of fresh and brackish water shrimp at day 10 exposure to Petroleum refinery oily sludgein comparison with GESAMP toxicity rating (1997).

Test shrimp	Duration	GESAMP (1997) Toxicity Rating for Damage to Living Resources			
		LC_{50} (mg/kg)	Rating (Days)	Toxicity status	96h LC_5
Fresh water shrimp	**10**	1097.375 ± 5 0.62	Very highly toxic	5	<0.10
Brackish water shrimp	**10**	1590.376 ± 0.92	Highly toxic	4	0.10-1.0
			Moderately toxic	3	1.0-10
			Slightly toxic	2	10-100
			Practically non-toxic	1	100-1000
			Non-hazardous	0	>1000

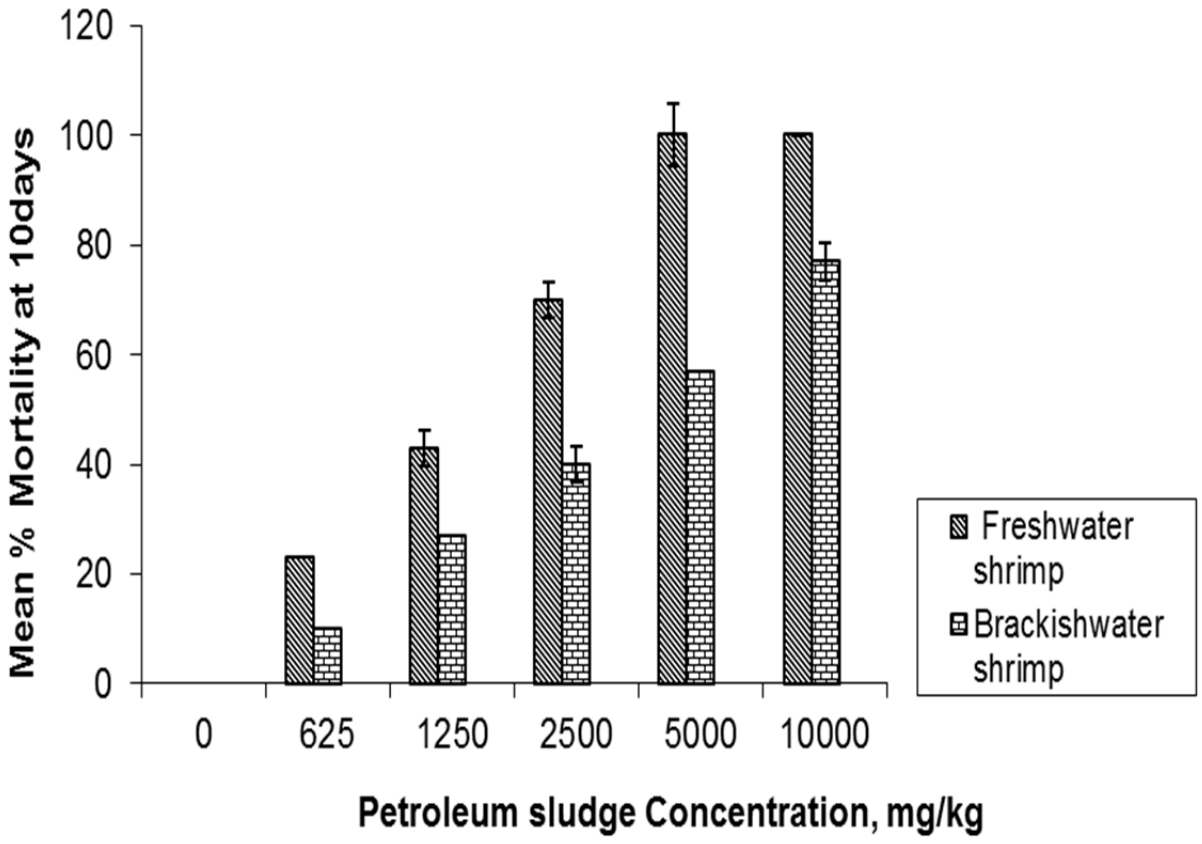

Figure 1. Mean percentage (%) mortality of shrimp exposed to petroleum refinery oily sludge after 10 days.

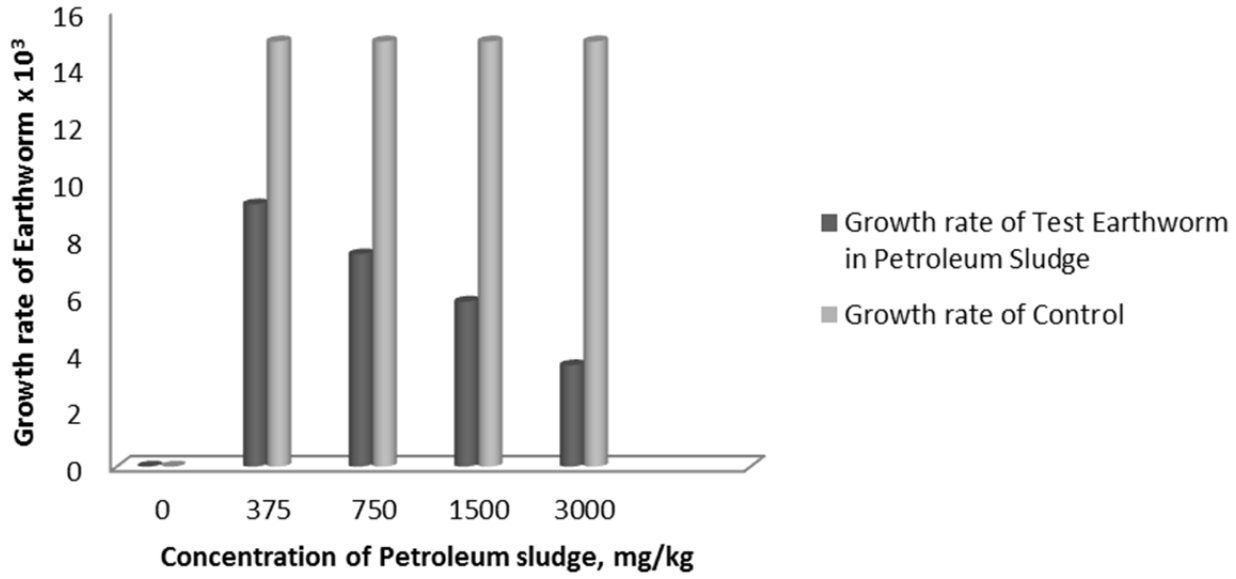

Figure 2. Growth rate of earthworm in petroleum refinery oily sludge.

Table 6. Chronic toxicity profile of Petroleum refinery oily sludge on the growth rate *of Apporectoda longa.*

Test sample	Time (days)	EC_{50}, mg/kg	Earthworm' Toxicity		
			Rating	Designation	EC50 (mg/kg)
Petroleum Sludge	7	Cannot be determined not up to 50% deaths	1	Super toxic	< 1.0
Petroleum Sludge	14	Cannot be determined not up to 50% deaths	2	Extremely toxic	1.0-10
Petroleum Sludge	21	1655	3	Very toxic	10-100
Petroleum Sludge	28	825.04	4	Slightly toxic	100-1000

Table 7. Bioconcentration factor of TPH in Earthworm exposed to Petroleum refinery oily sludge for 28 days

TPH concentration (mg/kg)	TPH concentration in Earthworm	BCF
375	1938	5.168
750	2052	2.738
1500	2660	1.773
3000	3651	1.2173

from the lowest to highest sludge concentration (Table 7). This indicates that the sludge would be bioaccumulated into the tissues of terrestrial organism as sludge concentration increases.

DISCUSSION

The discharge of untreated petroleum refinery oily sludge into the environment has been shown to have acute and chronic effects on the biotic and abiotic components of the aquatic and terrestrial environments (Wang and Reed, 1984; Ma and Ortolano, 2002; Tang et al., 2012). The acute effect of petroleum refinery oily sludge pollution on *Nitrobacter* sp. in the aquatic environment was conducted since the nitrification process is a function of enzyme activity and its measurement has been used as an indicator of pollution (Williamson and Johnson, 1981; Wang and Reed, 1984). The decline in the as an indicator of pollution (Williamson and Johnson, 1981; Wang and Reed, 1984). The decline in the *Nitrobacter* sp. counts as the concentration of petroleum refinery oily sludge increased could be due to the toxic effect of sludge

resulting from sludge concentration as earlier reported by Okpokwasili and Odokuma (1997).

The genus *Nitrobacter* belongs to a variety of nitrate-oxidizing bacteria which are responsible for the second step of the nitrification process (oxidation of nitrite to nitrate). This bacterium was used for the chronic toxicity test of petroleum refinery oily sludge on nitrogen transformation activities in soil. This second step of nitrification is particularly sensitive. Inhibition of this step under uncontrolled conditions may lead to accumulation of nitrite nitrogen which is toxic (Dokaniakis et al., 2005).

As stipulated in the test guideline, OECD TG 216 (2000), since the difference between the lowest and highest percentage inhibition is greater than 25%, the sludge has the potential to inhibit nitrogen transformation. The observed increase in inhibition of transformed nitrogen as the concentration of petroleum refinery oily sludge increased could be due to the increase of some physicochemical properties of sludge such as total petroleum hydrocarbon (TPH) and metals (Wilde et al., 1983; Okpokwasili and Odukuma, 1997). Wang and Reed (1984) noted that a high concentration of metal cations inhibits microbial activities by causing damage or inactivating one or more critical enzymes resulting in formation of an inactive complex between the metal cations and an active enzyme. TPH contains toxic compounds such as PAHs and these have also been implicated in the inhibition of nitrification process (Suschka et al., 1996; Dokaniakis et al., 2005).

The obtained LC_{50} values for the acute toxicity test of the test sample on the shrimp indicated that the freshwater test organisms were more adversely affected by the sludge than the brackish water shrimps. Buikema et al. (1982) observed that the higher the LC_{50} value, the lower the toxicity or sensitivity of the test organism and vice versa. The difference in the response of the fresh and brackish water shrimps may be attributed to the osmoregulatory demand of the different environments.

The reduction of growth at higher concentration for the chronic toxicity test of petroleum refinery oily sludge on earthworms showed it reduced growth progressively as the sludge concentration increased and could eventually lead to death. The mechanism of toxicity of hydrocarbon to earthworms was observed to be based on the ability of hydrocarbons to bind to the Polar Regions in biogeneous membrane and to disorganize them (Krab et al., 2000). MacGeer et al. (2003) recorded an inverse relationship between BCF and exposure concentration of the test chemical on earthworms and attributed this to the lipo-philic nature of the sludge.

Conclusion

In conclusion, the findings from this research indicate that petroleum refinery oily sludge if not properly treated before disposal into the recipient environment could pose serious threat to the physiological and reproductive functions as well as survival of aquatic and terrestrial organisms. Also, xenobiotic compounds such as PAHs that are bioaccumulated in tissues of aquatic or terrestrial organisms could move up the food chain in higher organisms such as man and lead to conditions such as cancer, infertility among others.

Conflict of interests

The authors did not declare any conflict of interest.

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REFERENCES

American Public Health Association (APHA) (1998). Standard, Methods for the Examination of Water and Waste Water 20th Edition Published by American Public Health Association, Washington DC. 1220p.

American Society for Testing and Materials (ASTM). Standard E 2172-01 (2001). Standard guide for conducting laboratory soil toxicity with nematode *Caenorhabditiselegans*. Annual Book of ASTM Standard 11: 1606-1616.

Atlas RM, Bartha R (1992). Hydrocarbon biodegradation and oil spill bioremediation; in KC Marshall (Ed). Advances in Microbial Ecology. New York. Plenum Pres. Vol. 12, pp. 287-338.

Buikema AL, Nederlehner BR, Cairn J (1982). Biological Monitoring Part IV – Toxicity Testing. Water Resources 16:239-262.

Colwell RR, Zambruski MS (1972). Methods of Aquatic Microbiology University Park Press, Baltimore. 461p.

Davies NA, Hodson ME, Black S (2003). The influence of time on lead toxicity and bioaccumulation determined by the OECD earthworm toxicity test. Environ. Pollut. 121(1):55-61.

Department of Petroleum Resources (DPR) (2002). Environmental Guidelines and Standards for the Petroleum Industry in Nigeria (EGASPIN*)* 314p.

Dokaniakis SN, Kornaros M, Lyberatos C (2005). On the effect of xenobiotic bacterial nitrite oxidation. Proceedings of the 9th International Conference on Environmental Sciences and Technology Rhodes Island, Greece.

Doube BM, Schmidt O (1997). Can the abundance or activity of soil macrofauna be used to indicate the biological health of soils? In: Biological Indicators of Soil Health. Eds. C. Pankhurst, B Doube and V. Gupta. CAB International New York. pp. 265-295.

Duffus JH (1980). Environmental Toxicology (Resource and Environmental Science Series). Arnold Publishers Ltd. 26p.

Edwards WM, Shipitalo MJ (1998). Consequences of earthworms in agricultural soils; Aggregation and porosity. In Earthworm Ecology (Eds C.A. Edwards) St. Lucie Press Boca Raton, Florida. pp. 147-161.

Englert CJ, Kenzie EJ, Dragun J (1993) . Bioremediation of petroleum products in soil, Principles & Practice of Petroleum Contamination, in soils. API, 40:111-129.

Environment Canada (1992). Biological Test Method; Acute test for

sediment toxicity using fresh and brackish or estuarine amplipods. Conservation and protection, Ottawa, Ontario Report EPSI /RM/26.
Finney DJ (1978). Probit Analysis, Cambridge University Press, London. 333p.
Grunditz C, Dalhammar G (2001). Development of nitrification inhibition assays using pure cultures of *Nitrosomonas and Nitrobacter.* Water Res. 35:433-440.
Hazardous Waste Management System (1997). Environmental Protection Agency 62:29-32.
Krab J, Mathes K, Breckling B, Lonker O, Schulz – Berend V (2000). Scale – up biologischer Reinigungsvertahren – projeckt – Abachlubericht, bmb .F; Bremen.
Ma X, Ortolano L (2002). Environmental regulation in China, Rowman and little field. Publishers, Inc . 209p.
MacGeer JC, Brix KV, Skeaff JM, Deforest DK, Brigham SI (2003). Inverse relationship between bioconcentration factor and exposure concentration for metals, implication for hazard assessment of metals in the aquatic environment. Environ. Toxicol. Chem. 22:1017-1037.
Mishra S, Jyot J, Kuhad RC, Lal B (2001). Evaluation of inoculums addition to stimulate in – situ bioremediation of oily – sludge contaminated soil. Appl. Environ. Microbiol. 67:1675-1681.
Okpokwasili GC, Odokuma LO (1997). Response of Nitrobacter to oilfield dispersants and domestic detergents. Trop. Freshw. Biol. 6:65-74.
Oolman T, Baker RR, Rentro NL, Marshall GE (1996). Refinery Uses Bioslurry Process to Treat RCRA Wasters. Hydrocarb. Process. 71(76):18-20.
Organization for economic co-operation and Development (OECD) (2002). Guidelines for testing of chemical TG 216. C. 21 Soil microorganisms; Test Nitrogen Transform.
Organization for economic cooperation and Development (OECI) (2004) Guidelines for the Testing of Chemicals No 218; Sediment – water chironomid toxicity test using spiked sediment (Adopted April 2004).
Prospt TL, Lochmiller RI, Quals CW, Mcbee K (1999). *In-situ* (mesocosm). Assessment of immunotoxicity risks to small mammals inhabiting petrochemical waste sites. Chemosphere 38:1049-1062.
Spiegel H (2002). Trace element accumulation is selected bioindicators exposed to emission along the industrial facilities of Danube, lowland. Turk. J. Chem. 26:815-823.
Suschka J, Mrowies B, Kuszmider G (1996). Respose of Nitrobacter toxicity of oil field dispersants and domestic detergents. Trop. Freshw. Biol. 6:65-74.
Tang J, Lu X, Sun Q, Zhu W (2012). Aging effect of Petroleum Hydrocarbon in Soil under different Attenuation Conditions. Agric. Ecosyst. Environ. 149:109-117.
Terhivuo J, Pankakoski E, Hyvarinen H, Koivisto K (1994). PB uptake by ecologically dissimilar earthworms (Lumbricidae) species near a lead smelter in Southern Finland. Environ. Pollut. 85:87-96.
United State Environmental Protection Agency (USEPA) (2004). United States Environmental Protection Agency, understanding oil spills and oil spill response. htt:// www.fundyforum.com/profile archives/ profile 3.html.
Ururahy AFP, Marins MDM, Vital RL, Therezinha I, Pereira N (1998). Effect of Aeration on Biodegradation of Petroleum Wastes. Rev. Microbiol. 29:4.
Wang W (1984) Time response of Nitrobacter to toxicity. Environ. Int. 10:21-24.
Wang W, Reed P (1984). "Nitrobacter bioassay for aquatic toxicity." Toxicity screening procedures using bacterial systems. 309-325.
Wilde EW, Soracce RJ, Mayack LA, Shealy RL, Broadwell TL, Steffen RF (1983). Comparison of chlorine and chlorine dioxide toxicity to fathead Minnows and Bluegill. Water Res. 17(10):1327-1331.
Williamson KJ, Johnson OG (1981). A bacterial bioassay for assessment of wastewater toxicity. Water Res. 15:383-390.