

Full Length Research Paper

Comparative study of the acute toxicity of petroleum sludge on fresh and brackish water shrimp

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The ecological impact of petroleum sludge on fresh water shrimp (*Desmoscaris trispinosa*) and brackish water shrimp (*Palaemonetes africanus*) in the Niger Delta area was compared using the Organization of Economic Co-operation and Development (OECD) No. 218 protocol for sediment toxicity test. The shrimp were exposed to sediments treated with petroleum sludge at concentrations of 625, 1250, 2500, 5000 and 10000 mgkg⁻¹. The LC₅₀ value obtained with *D. trispinosa* (1100 ± 160 mgkg⁻¹) was lower than that obtained with *P. africanus* (1590 ± 336 mgkg⁻¹). This was an indication that the petroleum sludge was more toxic to the fresh water shrimp than the brackish water shrimp. However, the observed mean LC₅₀ values were not significantly different at levels of $p < 0.01$. These values indicated that petroleum sludge can have adverse effects on the fauna in the shoreline/benthic sediment of the Niger Delta ecological zone. Consequently, there is the need to protect sensitive invertebrates representing the major proportion of the diet of many species and humans.

Key words: Toxicity, petroleum sludge, sediment, shrimp, bioassay.

INTRODUCTION

The discharge of toxic pollutants into fresh and marine environment presents risks to the biota, in particular; to sessile, bottom-dwelling organisms, unless contaminant concentrations remain below certain tolerable concentrations (Dickson et al., 1987). Sediments are known repositories for physical debris and "sinks" for a wide variety of chemicals, organics and metals, which tend to sorb to particles that eventually end up as bottom deposits; once there, they may become available to the overlying ecosystem, be transformed into more or less toxic forms, or they may bioaccumulate in benthic organisms (Dickson et al., 1987).

The concern associated with the chemicals sorbed to sediments is that many commercial species and food

chain organisms spend a major portion of their life cycle living in or on aquatic sediment and this provides a pathway for these chemicals to be consumed by higher aquatic life in the food chain, fish and wildlife, including avian species as well as humans (Burton, 1992).

Sediment toxicity testing has developed rapidly into a reliable and efficient tool for evaluating the biological risk of contaminants in sediments (Burton, 1992; Southerland et al., 1992). They provide important information that cannot be derived solely from chemical analysis or from community surveys (USEPA, 1994). The information generated from the sediment toxicity tests can be used to demonstrate the bioavailability of sediment contaminants, evaluate the toxic effects of various contaminants such

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demonstrate the bioavailability of sediment contaminants, evaluate the toxic effects of various contaminants such as metals, petroleum products and sludge, and toxic organic chemicals in the medium and also to evaluate the toxicity of substances whose biological effects may not have been well characterized or known (USEPA, 1994).

Petroleum sludge is an oily and viscous residue, which is formed during production, transportation and refining of petroleum and is composed of basically oil, water and solids (Ururahy et al., 1998). Chemically, the presence of aromatics, polycyclic aromatic hydrocarbons (PAHs), and complex compounds with a very high molecular weight, such as asphaltenes confer on sludges high recalcitrance (Ururahy, 1998). Toxic organics, radioactive materials and metals that are considered hazardous constitute 80% of petroleum wastes sludge (USEPA, 1993). They also possess a multiphase system that is very stable due to the adsorption of oil on solid particles, producing a highly protective layer (Hann and Loehr, 1992).

Sources of petroleum sludge include sediment and finely divided corrosion products that accumulate inside pipes, bottom of storage tanks and separators and produced water equipment. The sludge usually contain carbonates and silicates with a density of approximately 1.6 gcm^{-3} (USEPA, 1993; Testa et al., 1994).

Ecotoxicologically, some compounds of petroleum sludge act as solvents of microbial membranes thereby impairing the biodegradation potential of these microbes (Prince and Sambasiram, 1993). They could also clog fish and shrimp gills and feathers of aquatic birds leading to the death of these organisms. Many of the constituents of oily sludge are carcinogenic and potent immunotoxicants (Propst et al., 1999). Bioaccumulated constituents of the oily sludge move up the food chain and since they are recalcitrant, they can lead to mutations and cancers, among other conditions, in man and other higher organisms (Atlas and Bartha, 1992).

When petroleum sludge pollutes the environment, it affects aquatic species by altering essential elements of their habitat (National Geography, 2000). For example, petroleum spillage in water does not quickly dilute, but tends to remain in a concentrated mass on the surface, which is only slowly changed, and degraded (Peterson and Lubchenco, 2000). Thus, its most pronounced effects on aquatic organisms are on those who make use of the water surface or inhabit the shorelines/benthos (Brassard, 1996).

The loss of benthic organisms can obviously have negative consequences for an ecosystem through disruption of the food web (Bohannon et al., 2002). Many benthic organisms are very sensitive to toxic chemicals in pore water (Burton, 1992). These benthic species are particularly sensitive to water soluble contaminants, affecting the ability of the organisms to reproduce, avoid predators and feed (Burton, 1999; Bat et al., 1999). The suitability of shrimp for sediment toxicity testing is due to the fact that they are highly prolific, readily available year-

round and reach harvestable size in three to four months, if their growing conditions are favorable (Banks and Brown, 2002). They give a consistent, reproducible response to toxicants that is, they are appropriately sensitive. They are relatively easy to handle in laboratory conditions and are important ecologically and economically.

Shrimps play an important role in the coastal food web serving as an important nutrition source for small worms, sand fleas, shellfish, fish, birds, invertebrates and humans (Ciarelli et al., 1997; Bryant et al., 1984; Bryant et al., 1985). Anderson et al. (1997) stated that shrimp are used for biomonitoring of petroleum pollution because their dose and time-dependent accumulation are reflective of the levels of non-essential metals present in contaminated wetlands.

The aim of this study was to investigate the toxic effects of petroleum sludge on the aquatic ecosystem using fresh water shrimp (*Desmoscaris tripsinosa*) and brackish water shrimp (*Palaemonetes africanus*).

MATERIALS AND METHODS

Test organisms

The test organisms *D. tripsinosa* and *P. africanus* of the Nigerian Niger Delta ecological zone were collected from fresh and brackish water environments respectively. The shrimp from both environments were acclimated in their respective dilution water (that is, water from the habitat of the shrimp) in holding tanks (dimension length \times height \times width = $100 \times 100 \times 100 \text{ cm}$). Acclimation of organisms to laboratory conditions was for seven days prior to commencing the test. The shrimp were not fed for 24 h before test initiation (Reish and Oshida, 1986). The shrimp were considered acclimated when no mortality is recorded within a seven-day period (USEPA, 1994).

Test medium

Petroleum sludge was used as the test chemical for the 10-day sediment toxicity test. The constituents of the sludge were analysed according to the methods of USEPA (1984), API-R35, ASTM (1998) and APHA (2006). The sludge was collected from the sludge holding tank of Warri Refinery and Petrochemicals Company, Ekpan, Delta State, Nigeria. It was collected in 4-litre plastic cans and stored at 4°C until required for use.

The top few centimeters of sediments from fresh and brackish water environments were sampled by a hand held Van Veen grab. After collection, the sediment was sieved ($500 \mu\text{m}$ mesh) to remove large objects and any organisms, which may interfere with the test. The sediment was allowed to settle overnight and the supernatant 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, after which 1 kg was weighed into each tank (Whale and Worden, 1999).

Bioassay procedure

Experimental procedure for the 10-day sediment toxicity test was conducted in accordance with the procedures detailed in No. 218

sediment toxicity test protocol (OECD, 2004). A preliminary range-finding test was conducted prior to the definitive test to assist in determining the appropriate dilutions to be used for the test. The range-finding test was conducted using a broad concentration range (200, 2000 and 20000 mgkg⁻¹) 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 test and were in appropriate logarithmic dilution series.

The concentrations of the sludge used for the definitive test were 625, 1250, 2500 and 5000 and 10000 mgkg⁻¹ in three replicates. The 10-day static sediment bioassay with renewal of the overlaying water was conducted by placing the weighed sediment into triplicate sets of 5 L amber coloured glass tanks. The sediment in the container was spread evenly and 2 L of the prepared test solution was gently added. The contents of the containers were then left to settle for 2 to 3 h prior to the addition of the test organisms. Shrimp were sieved through a 500 µm mesh sieve and placed in dilution water to rinse off any debris. Ten shrimp were gently transferred into each glass vessel containing the sludge and control. The overlaying test solutions were decanted every 2 days and replaced with two liters of freshly prepared test solutions to ensure same concentration for the test duration. It was then gently aerated by passing air through air stones attached to electrical pumps, for the 10-day exposure period.

Observations for mortality in the test vessels was carried out and records made of the numbers of shrimp which were swimming, crawling on the surface, loss of appendages, emergence of organisms from sediment, immobilized (lying on the sediment surface but obviously still alive) or dead (ASTM, 1992; Aqua Sense, 2004). Dead animals were removed at each observation. After, 10 days the sediments were sieved and the number of shrimp recorded. Average mortality in the bioassay that is, the total number of dead organisms related to the total number of organisms used on day 0 was used to estimate the average mortality in the bioassay at day 10.

Control test

To prove the quality and responsiveness of the shrimp used in the sediment test, controls with untreated sediment was conducted. The toxicity was valid if control mortality was <10% (Buikea, 1982).

Laboratory and data analysis

Water quality with respect to such parameters as pH, temperature, total dissolved solids, ammonia, dissolved oxygen and total petroleum hydrocarbon (TPH) and PAHs were measured by standard methods as stated in APHA (1998). The mean temperature during the experimental period in all bioassays was 27 ± 2°C with a 16:8 h light: darkness photoperiod. The susceptibility of the test shrimp to petroleum sludge in both the fresh and brackish water 10-day sediment toxicity test was determined using the Finney Probit analysis (Finney, 1971). Computations of confidence interval of mortality rate were also obtained from the analysis used to determine the LC₅₀. The paired t-test analysis of variance (ANOVA) was used to test the variables at $p < 0.05$ level of significance and this was done with the Statistical Package for Social Science (SPSS) statistical software Version 16.0. Bar charts were also used in this study for the pictorial representation of the assessment endpoint.

RESULTS

The values of the physicochemical parameters analysed

in the petroleum sludge are presented in Table 1. The sludge was acidic as was indicated by the pH value of 5.81±0.28 while the concentration of TPH was high (340,000 ± 50,000 mgkg⁻¹). PAH recorded 0.075 mgkg⁻¹ while the value for ammonium was 21.7 mg l⁻¹. The mean toxicity profile of the fresh and brackish water shrimp exposed to varying concentrations of petroleum sludge in the sediment are presented in Table 2. The results showed that no death or physiological changes were observed in the negative controls for the 10-day test duration.

The control shrimp appeared active and healthy (responsive to stimuli) throughout the test period. The shrimp exposed to the various sludge concentrations had higher mean % mortality on day 10 in the fresh water test (100%) than in the brackish water test (93%) (Figure 1). The results obtained indicate that mortality increased with increased sludge concentrations and exposure duration. The LC₅₀ for the fresh water shrimp was 1100 mgkg⁻¹ ± 160 while that for the brackish water shrimp was 1590 mgkg⁻¹ ± 336 (Figure 2). Observed percentage mean mortality of the test shrimp in both the fresh and brackish water environments in relation to the control in a one-way ANOVA test were not significantly different at $p = 0.1016$, $F = 2.492$.

The concentration of the physicochemical parameters of the overlaying solutions in each of the test tanks in the fresh water and brackish water shrimp toxicity experiment are shown in Tables 3 and 4 respectively. Results obtained showed there were no much difference in values obtained for both the fresh water and brackish water test solutions except for the value obtained for TDS in the brackish water test solutions which were much higher than that of the fresh water. Physiological changes were associated with exposure to the sludge in both the fresh and brackish water environments especially at high concentrations. Some surviving individuals emerged out of the sediment showing signs of stress and swam erratically in the overlying water. Some of the organisms on the sediment surface were immobile. Cephalothoraxes were broken from the abdomen of most of the dead organisms, while discoloration of the dead organisms was also noticed especially in the higher concentrations. Other behavioural changes were observed at high concentrations for shrimp. These include excitement, increased activity, and scattering in the water.

DISCUSSION

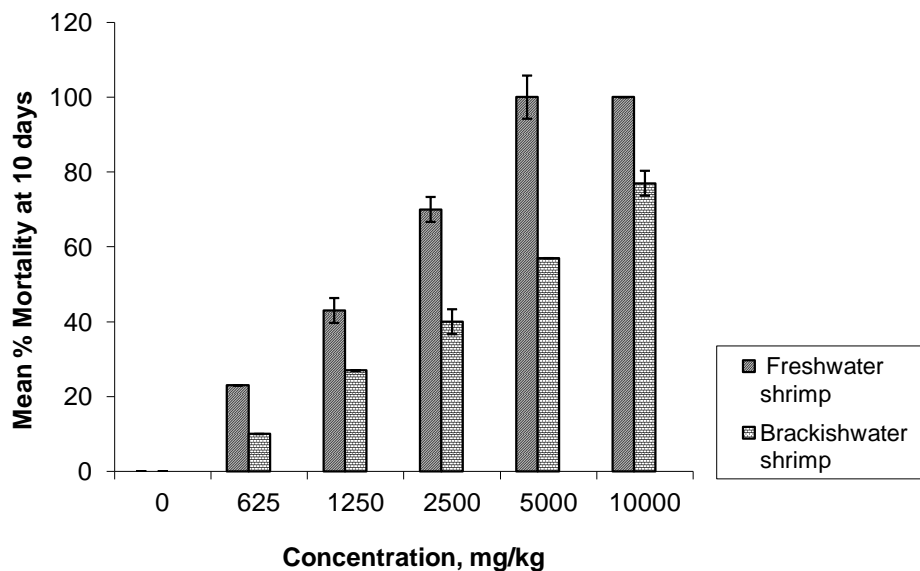
The values obtained for the LC₅₀ indicates that the fresh water shrimp *D. trispinosa* were more sensitive to the petroleum sludge than the brackish water shrimp *P. africanus*. Buikema et al. (2000) observed that the higher the LC₅₀, the lower the toxicity to the test organisms and vice versa. The difference in response may be related to the relative activity levels of the species tolerance in the

Table 1. The physicochemical and microbiological characteristics of petroleum sludge

Parameter	Mean (\pm S.E)
pH	5.81 \pm 0.28
Conductivity, us/cm ²	466.65 \pm 25.25
Sulphate, mg/kg	4.83 \pm 0.64
Nitrate, mg/kg	26.40 \pm 1.02
Phosphate, mg/kg	7.73 \pm 0.88
Total Nitrogen, mg/kg	0.12 \pm 0.50
Total Petroleum Hydrocarbon, mg/kg	340,000 \pm 50,000
Polyaromatic Hydrocarbon, mg/kg	0.075 \pm 0.02
Ammonium, mg/kg	21.65 \pm 1.21
Copper, mg/kg	5.53 \pm 0.20
Chromium, mg/kg	8.68 \pm 0.03
Nickel, mg/kg	3.36 \pm 0.02
Cadmium, mg/kg	0.32 \pm 0.50
Zinc, mg/kg	100.65 \pm 2.30
Barium, mg/kg	0.31 \pm 0.04

Table 2. Acute toxicity profile of fresh and brackish water shrimp exposed to petroleum sludge at day 10.

Parameter	Fresh water	Brackish water
LC ₅₀ , mg/l ⁻¹	1097.375 \pm 0.62	1590.376 \pm 0.92
Confidence limit	378.25 – 1835.894	27.194 – 4387.799
Probit equation	Y = 3.22 + 1.82 logx	Y = 3.52 + 1.25 logx
Slope	3.571 \pm 0.27	10.408 \pm 0.00

**Figure 1.** Mean % mortality \pm SE of fresh and brackish water shrimp exposed to petroleum sludge at day 10.

brackish environment and the toxicant's mode of action (Bury et al., 1999). Similarly, the relative difference observed in the mean % mortality and 10-day LC₅₀

values between the fresh and brackish water test may not be unconnected with the varying osmoregulatory demand of the different environment. It has been reported that in

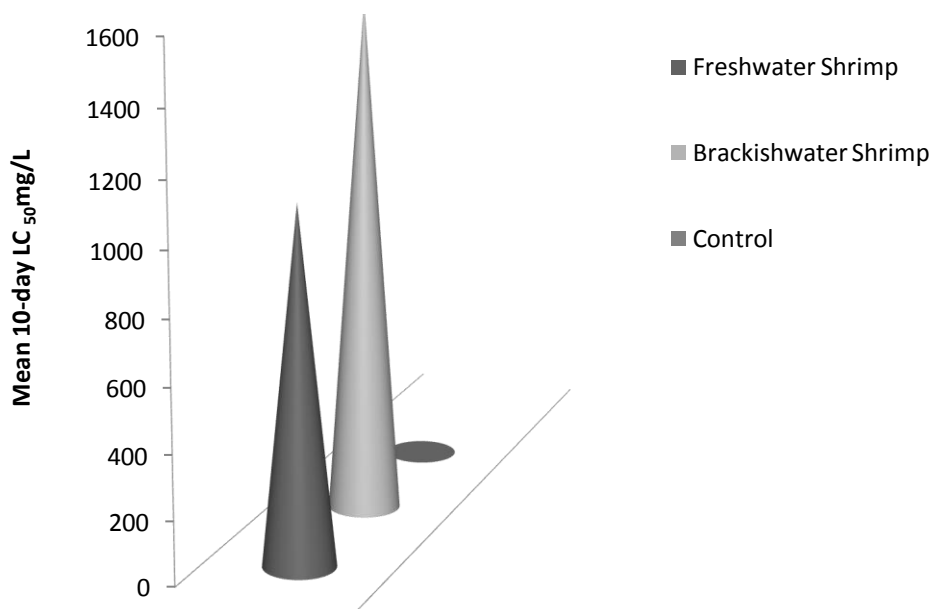


Figure 2. Mean LC₅₀ of fresh water and brackish water shrimp exposed to petroleum sludge.

Table 3. Mean concentrations of physicochemical parameters of overlaying test solution for fresh water shrimp exposed to petroleum sludge.

Parameter	Concentration of sludge in test solutions (mg/L)					
	Control	625	1250	2500	5000	10000
TPH, mg/L	<1.00	3.64 ± 0.02	5.43 ± 0.06	6.75 ± 0.01	8.66 ± 0.01	11.77 ± 0.01
DO, mg/L	6.17 ± 0.06	5.73 ± 0.07	5.43 ± 0.06	4.83 ± 0.08	4.53 ± 0.08	4.27 ± 0.06
pH	7.35 ± 0.02	6.56 ± 0.01	6.45 ± 0.01	6.33 ± 0.02	6.23 ± 0.01	6.16 ± 0.02
TDS, mg/L	49.27 ± 0.15	65.69 ± 0.02	81.17 ± 0.06	88.60 ± 0.02	93.70 ± 0.04	97.10 ± 0.03
Ammonium, mg/L	0.46 ± 0.01	2.48 ± 0.02	3.24 ± 0.01	4.77 ± 0.01	5.40 ± 0.02	6.68 ± 0.02

Table 4. Mean concentrations of physicochemical parameters of overlaying test solution containing brackish water shrimp exposed to petroleum sludge.

Parameter	Concentration of petroleum sludge in test solutions (mg/L)					
	Control	625	1250	2500	5000	10000
TPH, mg/L	<1.00	4.60 ± 0.02	5.18 ± 0.03	6.28 ± 0.04	7.36 ± 0.02	10.29 ± 0.06
DO, mg/L	6.30 ± 0.10	5.73 ± 0.06	5.37 ± 0.06	5.0 ± 0.10	4.63 ± 0.12	4.23 ± 0.06
pH	7.68 ± 0.02	7.08 ± 0.03	6.88 ± 0.02	6.61 ± 0.01	6.40 ± 0.02	6.36 ± 0.04
TDS, mg/L	2208 ± 2.52	2260 ± 2.0	2271 ± 3.06	2291 ± 2.31	2239 ± 1.15	2423 ± 5.77
Ammonium, mg/L	0.31 ± 0.02	2.14 ± 0.03	2.64 ± 0.04	3.86 ± 0.04	4.75 ± 0.05	5.19 ± 0.06

the fresh water environment, any physical damage to external tissues of the organisms allows more water to enter the body, (and salt to escape), placing an additional burden on the kidneys, ultimately resulting in death (Bury et al., 1999). In the same vein, the buffering effect of the

brackish water environment as is indicated in a higher pH range than the freshwater environment could also have contributed to the observed lower effect on the brackish water shrimp. It has also been reported that toxicity of chemicals can be altered by variations in water chemistry

by affecting the amount of the chemical available to bind to the organisms and sediment particles (Bury et al., 1999; Adams et al., 1992). There was however a progression of effect from low mortality to high mortality with increase in toxicant values or concentrations in both environments. This could be attributed to the increase in the concentration of the physicochemical parameters of the overlaying solutions on the sediment as the concentration of the sludge increased (Ma and Ortalano, 2002). They stated that numerous physicochemical environmental factors including temperature, nutrient concentrations, dissolved oxygen (DO), Total Suspended Solids (TSS), pH, ammonia, salinity and composition of the test chemical influence the toxicity of chemical to aquatic organisms.

These parameters are essential to monitor during hydrocarbon pollution in an ecosystem since they could adversely affect the survival of the organisms used as biomonitors. The concentration of hydrogen and hydroxyl ions in water cause many chemicals to be absorbed in crustacean (NRC, 2002). Most of the pH values obtained in both the fresh water and brackish water overlaying solutions were acidic and could have contributed to the toxicity of the petroleum sludge.

At high concentrations, shrimp in both the freshwater and brackish water test were observed to be immobilized. Although mortality is the most commonly used toxicity test endpoint, immobilization is also ecologically relevant. The environmental consequences of immobilization following exposure to a toxicant are severe. Immobile animals would be incapable of maintaining their position in the sediment as well as the overlaying water column, would not be able to feed and avoid predators (Willis and Ling, 2003). Other behavioural changes observed at high concentrations by the shrimp such as excitement, increased activity, and scattering in the water was also observed by Stanislay (2000).

Petroleum sludge composition could account for its toxic effects on the shrimp in both aquatic environments, which include the presence of aromatics, polycyclic aromatic hydrocarbons (PAHs) and complex compounds with a very high molecular weight, such as asphaltenes which confer on sludges high recalcitrance (Ururahy et al., 1998). PAH concentration of the test sludge recorded 0.075 mg/kg. This could also have contributed to the toxicity of the sludge as observed by Samet et al. (2000) who reported that dissolved polycyclic aromatic hydrocarbons are toxic to aquatic species in concentrations usually ranging from 0.1 to 0.5 ppm.

Also it was observed that the PAH concentrations have an increasing trend with dose; continuous exposure over time eventually leads to the death of crustaceans (Barron et al., 2001). PAHs with a high molecular weight may cause sublethal effects; such as growth reduction, chronic diseases, reproductive impairment, at very low concentrations in biota: (5 to 100 ppb) in the tissue of the animal. Concentrations of PAHs in the aquatic environ-

ment are generally highest in sediment, intermediate in biota and lowest in the water column (CCME, 1992).

In water, PAHs attach to sediment, impacting bottom-dwelling organisms like periwinkle, shrimp, oysters and plankton. As these organisms spend time in or near contaminated sediments, they accumulate PAHs in their tissues leading to harmful effects. A wide range of PAH-induced ecotoxicological effects in a diverse suite of biota, including microorganisms, aquatic biota, amphibians and terrestrial mammals have been reported (Delistraty, 1997; Ekundayo and Benka-Coker, 1994; Khan and Law, 2005).

Toxic organics, radioactive materials and heavy metals that are considered hazardous constitute 80% of petroleum wastes sludge (USEPA, 1993) and could also have accounted for the sludge toxicity. Animals such as shrimp may be exposed to petroleum compounds by inhalation, direct contact with the skin, or ingestion. In addition to outright toxicity, the threat posed to aquatic species by the persistent residues of spilled petroleum sludge as emulsions in water is one of physical smothering (Brassard, 1996).

In the same vein, petroleum sludge pollution in the environment can affect organisms by direct physical coating, altering essential elements of the habitat, and by the direct toxic effects of chemicals in the petroleum sludge (National Geographic, 2000). It also rapidly penetrates into the species through gills and disturbs the body systems such as respiration, nervous system, blood formation and enzyme activity. The occurrence of this disturbance leads to a number of common symptoms like behavioral change and loss of oxygen due to the sludge pollution (Hosmer et al., 1998).

The biological effects of petroleum sludge on crustaceans influence behavioral performance and largely affect ecosystems. Ma and Ortalano (2002) reported that shrimp ability to recover normal behavior after exposure of petroleum decreases with increasing concentration and time. The effects of petroleum pollution on crustaceans are largely determined by the proportion of toxic components, the duration of oil exposure as well as the degree of other stresses (NRC, 2002).

Ammonium in the overlaying solutions in both environments tested was relatively high and its known to be toxic to aquatic lives at increased concentrations; its toxicity being complicated by temperature and pH (Lawson et al., 1995).

Wetlands are one of the nature's richest habitats providing food, water, and cover for a diversity of aquatic species. When petroleum sludge pollution reaches wetlands it causes extensive damage to aquatic species and vegetation. The impact of petroleum sludge pollution on wetlands is compounded by toxicity and tainting effects resulting from the chemical composition of petroleum sludge as well as diversity and variability of biological systems and their sensitivity to petroleum pollution (Bohannon et al., 2002).

Conclusion

The petroleum sludge was found to have a lower LC₅₀ in the test with fresh water shrimp when compared to that with brackish water shrimp. Since toxicity is inversely proportional to LC₅₀, the petroleum sludge had a relatively higher effect on the fresh water shrimp than the brackish water shrimp. However, although toxicity of the petroleum sludge was relatively lower in the brackish water environment, that mortality was observed is an indication of adverse effects of the sludge in the brackish water environment in the advent of an enormous spill. Also the insignificant difference between the LC₅₀ and standard deviations of both aquatic environments indicate that the sludge would produce similar adverse effects irrespective of the environment it pollutes, although with slight variations due to the differences in the physicochemical characteristics and environmental factors of both environments which could predispose shrimp and other aquatic fauna to sludge toxicity. Petroleum sludge needs to be properly treated and disposed to ensure that sensitive and important ecological organisms are not adversely impacted. Results of this bioassay could also be used as a regulatory and predictive tool to assess the health of the aquatic ecosystem around current dump sites where petroleum sludge is being indiscriminately dumped without appropriate treatment.

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Abbreviations: **CCME**, Canadian Council of Ministers of the Environment; **DO**, Dissolved oxygen; **DPR**, Department of Petroleum Resources; **EPA**, Environmental Protection Agency; **GC/MS**, Gas chromatograph-mass spectrophotometer; **LC₅₀**, Lethal Concentration 50; **OECD**, Organization for Economic Cooperation and Development; **PAHs**, Polyaromatic hydrocarbons; **PCBs**, Polychlorinated biphenyls.

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