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Eco-toxicity of petroleum hydrocarbon contaminated soil

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Abstract

Total petroleum hydrocarbons (TPH) contaminated soil samples were collected from Shengli Oilfield of China. Toxicity analysis was carried out based on earthworm acute toxicity, plant growth experiment and luminescent bacteria test. The soil was contaminated by-petroleum hydrogcarbons with TPH concentration of 10.57%. With lethal and sub-lethal rate as endpoint, earthworm test showed that the LD_{50} (lethal dose 50%) values in 4 and 7 days were 1.45% and 1.37% respectively, and the inhibition rate of earthworm body weight increased with higher oil concentration. TPH pollution in the soil inhibited seed germination in both wheat and maize experiment when the concentration of petroleum was higher than 0.1%. The EC_{50} (effective concentration 50%) for germination is 3.04% and 2.86% in maize and wheat, respectively. While lower value of EC_{50} for root elongation was to be 1.11% and 1.64% in maize and wheat, respectively, suggesting higher sensitivity of root elongation on petroleum contamination in the soil. The EC_{50} value in luminescent bacteria test was 0.47% for petroleum in the contaminated soil. From the experiment result, it was concluded that TPH content of 1.5% is considered to be a critical value for plant growth and living of earthworm and 0.5% will affect the activity of luminescent bacteria.

Key words: toxicity; total petroleum hydrocarbon; soil; earthworm; contamination

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Introduction

Although pollutants and its concentration in soil can be characterized rapidly by chemical method, the overall soil quality can not be reflected by only chemical analysis. Concerning on the eco-toxicity of pollutants, biological method is more suitable in determining possible hazard of pollutants in soil on the ecology and environment. However, efforts to conduct site specific risk assessments using ecotoxicity test and correlation to contaminant concentration is limited and relatively unsuccessful for hydrocarbon contamination (Dorn and Salanitro, 2000).

Toxicity assessment of the contaminated soil with bioassays could provide meaningful information regarding a characterization procedure in ecological risk assessment (Al-Mutairi et al., 2008). The ecological toxicity diagnosis includes different methods by using soil animal and plant as testing approaches. Earthworm was used as test animal in soil toxicity experiments and has been established as standard method by International Organization for Standardization (ISO) in 1993 (ISO11268-1). Toxicity of heavy metals on earthworm has been widely studied, and the research has been conducted in subcellular level (Huang et al., 2009; Klok and Thissen, 2009; Owojori et al., 2008). Study showed that PAHs impaired glucose metabolism, with an associated increase in fatty acid metabolism and changes in tricarboxylic acid (TCA) cycle intermediates in earthworm (Jones et al., 2008). Plant toxicity test includes root elongation, seed germination and plant growth test, which has also been used as standard method by ISO11269-1 (1993) and Organization for Economic Co-operation and Development (OECD, 2000). Study shows that most plants appear to show some sensitivity to the pollutants (Banks and Schultz, 2005). In addition, there are also reports on total pertroleum hydrocarbons (TPH) toxicity research based on *Microtox* test (Chaineau et al., 2003). Extraction of organic contaminants from the soil is a critical factor in *Microtox* test. Different extracts such as DMSO (dimethyl sulfoxide)/H_2O and DCM (dichloromethane)/DMSO have been used as extraction solution to evaluate the microbial toxicity of organic contaminant by *Microtox* test (Plaza et al., 2005).

Petroleum hydrocarbons are complex mixture of organic components with different molecular weights. It was suggested that petroleum components with lower $\log(K_{OW})$ had greater toxic potential than those with higher $\log(K_{OW})$ (Di Toro et al., 2007). The toxicity of petroleum hydrocarbon is strongly correlated with the lower boiling-point fractions and especially to those within the C10–C19 range (Brils et al., 2002; Jonker et al., 2006; Neff et al., 2000).

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The change of petroleum composition during bioremediation process will result in the change of biological toxicity of the soil. Hydrocarbons inhibited microbial biomass, and the greatest negative effect was observed in the gasoline-polluted sandy soil (Labud et al., 2007). Some of metabolic compounds of TPH such as aromatic ketones, aldehydes, carboxylic acids, fatty acids, esters, also contribute to the biota toxicities of petroleum pollutants (Lee, 2003). Although toxicities of crude oil in water and on aquatic organisms have been studied (Couillard et al., 2005; Perkin et al., 2005; Martinez-Jeronimo et al., 2005), the toxicity of crude oil in soil has seldom been evaluated. In addition, different test methods on toxicity reflect toxicity from only one aspect that may be not enough. Holistic toxicity experiments were carried out in this study to test the TPH toxicity in soil for a better evaluation of bioremediation process and eco-safety of TPH.

1 Materials and methods

1.1 Experimental materials and determination of TPH

The soil used in the experiment was collected from Binnan oil production plant of the Shengli Oilfield in Shandong Province of China. The soil was air dried with plant residues, stones and other debris excluded, and passed through 2 mm sieve. The properties of TPH contaminated soil from Shengli Oilfield can be seen in Table 1. Due to improper operation or accident caused by leakage and other long-term accumulation of petroleum pollutants, the soil TPH content is as high as 10.57%, causing serious pollution. The value of total N (TN) and total P (TP) are in the moderate rate as compared to the reported result (Li et al., 2010; Dong et al., 2003). Levels of heavy metals Ni, Cu, Cd, Pb, Cr were significantly lower than the national standards. Constituents of the petroleum hydrocarbons are as the following: saturated hydrocarbons 50.5%, aromatic hydrocarbons 20.8%, asphaltene and polar fraction 27.7% as detected by fractionate analysis.

Artificial soil was prepared based on the ISO standard: 10% of moss peat (pH 6, Tianjin Damao Chemical Reagent Factory, China); 20% of the kaolin clay (Northern Hospital Chemical Reagent Factory of Tianjin, China); 69% of the industrial quartz sand (including 50% of small particles 0.05–0.2 mm, Jiangtian Chemical Co. Ltd., China); 1% of calcium carbonate (Northern Hospital Chemical Reagent Factory of Tianjin, China).

Table 1 TPH content and other properties of petroleum contaminated soil

Index	Value	National Soil Standard (III)
pH	7.9	/
TPH (%)	10.57	/
TN (g/kg)	2.75	/
TP (g/kg)	0.11	/
Cd (mg/kg)	ND	1
Ni (mg/kg)	6.5	200
Cr (mg/kg)	12	300
Cu (mg/kg)	16.5	400
Pb (mg/kg)	ND	500

ND: not detected under detection level of 1 mg/kg; /: items not regulated.

Plant seeds were purchased from Shandong Academy of Agricultural Sciences, China. The germination rate of is more than 95%, plant seeds include wheat (*Triticum aestivum*), maize (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), tall fescue (*Festuca arundinacea*) and Mexican corn grass (*Euchlaena mexicana*). Earthworm (*Eisenia foetida*) was purchased from Tianjin Jialiming Earthworm Culturing Base at Ninghe County of Tianjin, China. It was acclimated for about 2 weeks in laboratory condition before use.

TPH content was determined by gravimetric analysis. Air-dried soil samples (5 g) were treated by ultrasound extraction with 15 mL chloroform for 15 min and the supernatant was collected. The extraction was repeated three times and the extracts were then concentrated to dryness with a rotary evaporator at 40°C. The TPH content was determined by gravimetric analysis. The recovery rate of the result was (99.70 ± 0.95)%.

1.2 Earthworm acute toxicity test

Toxicity of TPH on earthworm in artificial soil experiment was based on ISO method. The following six TPH contents were set in artificial soil: 5%, 3%, 2%, 1.5%, 1%, 0.5% by diluting 10.57% TPH contaminated soil with artificial soil, artificial soil and clean soil in the same site of the contaminated soil were used as control. Soil was prepared and then put in the brown bottle with 500 g for each. Humidity was adjusted to 60% of the maximum water holding capacity. Health earthworms of 300–400 mg weight were selected, washed with distilled water, and dried with filter paper. Ten earthworms were weighed as a group, and the average weight value was calculated. The earthworm was put into brown bottle soon after weighing, and the bottle was sealed with gauze. The number of earthworm death was checked after 4 and 7 days. The basis for the death of earthworms was determined by no response to acupuncture. After the experiment, the earthworms were weighed, and average value was calculated. Triplicate experiments were conducted for each TPH concentration.

1.3 Plant germination and root elongation inhibition

Plants were cultivated with 10.57% TPH contaminated soil. Quartz sand with particle size ≤ 2 mm was used as control. Soil of 300 g was put into 180 mm diameter petri dishes with the moisture adjusted to 50% of maximum water holding capacity, and 20 seeds was put in the soil. The experiment was carried out in an artificial climate chamber of 25°C under dark condition. The germination rate was measured after 4 and 10 days and plants with higher germination rate were selected for the following experiment.

Plant growth experiment at different TPH concentrations was carried out based on ISO and OECD method. Wheat and maize seeds were used. The contaminated soil is set to five concentrations: 0.1%, 0.5%, 1%, 2%, 3% by diluting 10.57% TPH contaminated soil with quartz sand (diameter ≤ 2 mm), and quartz sand was used as control. The quartz sand showed no difference on plant germination

with the clean soil in the same site of the contaminated soil in a preliminary experiment. Soil of 300 g was put in 180 mm diameter petri dishes, with 20 seeds added. Water was added to adjust the moisture to 50% water holding capacity. The experiment was carried out in an artificial climate chamber of 25°C under dark conditions. Germination rate and root length were measured after 72 hr and 4 duplicates were carried out.

1.4 Luminescent bacteria test on the petroleum contaminated soil

Air-dried soil sample of 5 g was used and extracted by ultrasound with dichloromethane (DCM). After filtration, DCM was removed by rotary evaporation and soil extract was concentrated to 5 mL. Then 5 mL dimethyl sulfoxide (DMSO) was added and concentrated to 5 mL. The determination of biological toxicity of the soil extract was conducted using luminescent bacteria of *Photobacterium phosphoreum* T3 according to national standard of China (GB/T 15441-1995). Freeze-dried luminescent bacteria powder was purchased from the Institute of Soil Science, Chinese Academy of Sciences, Nanjing, China. Triplicate experiments were conducted.

2 Results

2.1 Earthworm acute toxicity test

LD_{50} (lethal dose 50%) value was calculated based on weighted linear regression. The probability unit value was calculated based on Gaussian distribution table, and the result of 4 days LD_{50} calculation is shown in Fig. 1a. A good linear correlation was found between the doses and probabilities, and LD_{50} of 4 days was calculated based on Fig. 1a as 1.45%.

According to the regression equation in Fig. 1b, it can be obtained that LD_{50} of 7 days was 1.37% suggesting that the endurance of earthworms to the oil pollution decreased after 7 days as compared to that of 4 days. All the earthworms died after 7 days when the TPH content exceeded 3%. The death rate was 90% at 7 days when the TPH content was 2%. At TPH content of 0.5%, the survival of earthworms was not affected even after 7 days. This result is in consistence with that of Hanna and Weaver (2002).

Body weight of earthworms was tested on the survival treatment group of TPH ≤ 1.5% and the result is shown in Fig. 2. Earthworm weight in the control group also decreased which may be related to changes in living conditions and reduced soil nutrients. With the increase in TPH content, inhibition rate of earthworm weight increased up to 48.91% at TPH content of 1.5%. Pollutant concentrations and inhibition rate of body weight was positively correlated and showed significant dose-effect relationship which can be regarded to be caused by the stress from petroleum hydrocarbons and leads to a high rate of weight suppression.

2.2 Inhibition of plant germination and root elongation

Five kinds of plant seeds: wheat, maize, cotton, Mexican corn grass and tall fescue were tested, respectively, at the 4 and 10 days of germination. As shown in Table 2, germination of maize and wheat has high tolerance to TPH contamination. Under the high content of contaminated

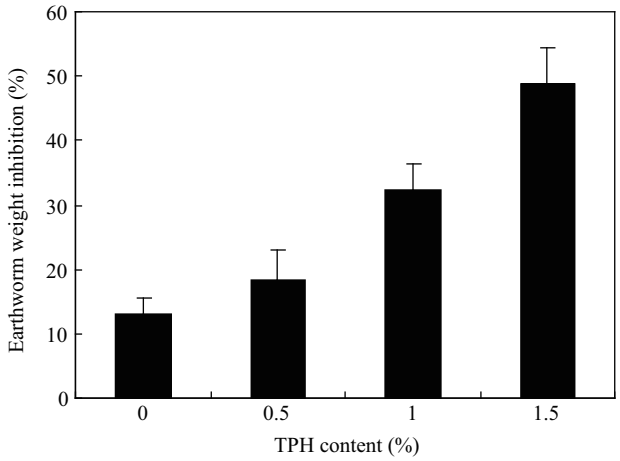

Fig. 2 Inhibition rate of earthworm body weight for 7 days in different TPH contaminated soils.

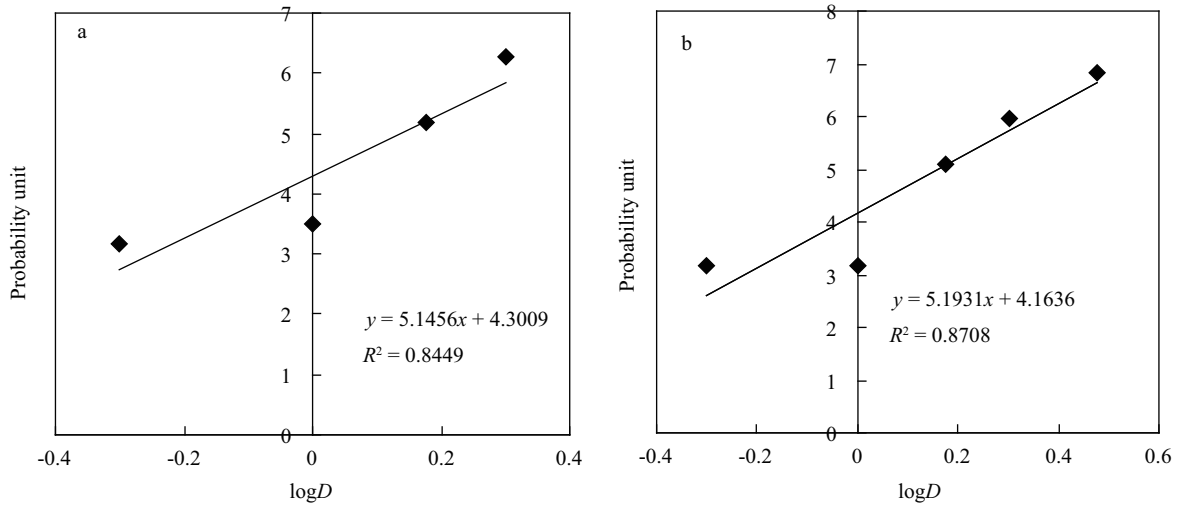

Fig. 1 Regression analysis of LD_{50} for 4 (a) and 7 days (b) in different TPH contaminated soils. *D*: soil TPH content.

Table 2 Comparison of seed germination of five different plant species after 4 and 10 days in TPH contaminated soil (%)

Plant	4 days germination	4 days control	10 days germination	10 days control
Wheat	20	95	60	100
Maize	10	90	45	100
Cotton	0	0	0	10
Mexican corn grass	0	30	0	75
Tall fescue	0	95	10	100

soil, the 4 days germination rates were 10%, 20% and 10 days germination rates were 45%, 60% for maize and wheat, respectively. Tall fescue is the next that can germinate in the contaminated soil; however, the germination rate was only 10% after 10 days. Mexican corn grass and cotton did not germinate.

Based on above results, wheat and maize were selected for further test on the germination and growth experiment. The 72 hr germination and root length inhibition of maize are shown in Fig. 3. It can be seen that 0.1% TPH contaminated soil inhibited the germination of maize with a value of only 7.69%. When the TPH content reached 3%, the germination inhibition rate was 51.28%. The 0.1% TPH contaminated soil did not inhibit root length of maize, on the contrary, slightly stimulation on the root elongation was found. The inhibition was significant starting from 0.5% of TPH contaminated soil, and rapid increase of inhibition was found between TPH content of 0.5% and 1%, with increasing extent of 27.61%. The inhibition rate at TPH content of 1% is 66.59%, which has exceeded 50% and means a significant effect on the maize growth. At TPH content of higher than 1%, the inhibition on root growth increased with high content but with smaller extent. The inhibition rate reached 81.51% at TPH content of 3%. The EC_{50} (effective concentration 50%) values on germination and root elongation for maize are 3.04% and 1.11%, respectively, indicating a higher sensitivity of root elongation on TPH contamination in soil.

Figure 4 shows inhibition rate of wheat germination and root elongation in 72 hr by different TPH contaminated soil. No inhibition was found in 0.1% TPH. The inhibition rate on germination increased with elevated TPH content. The inhibition rate was 48.38% at TPH content of 3%, not exceed 50% suggesting strong tolerance of wheat on TPH. However, all concentrations of TPH contaminated soil showed inhibition on the wheat root length, the inhibition

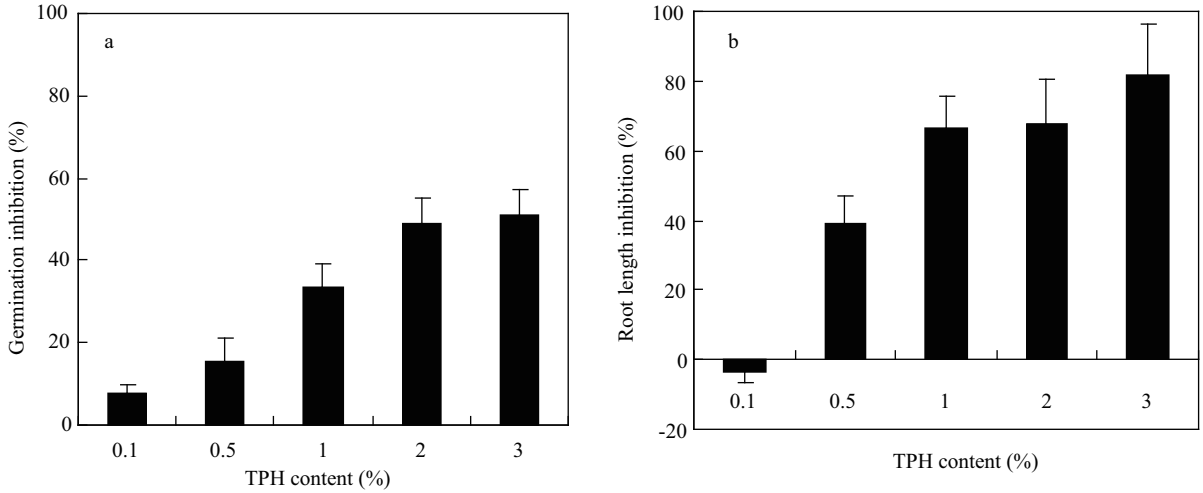

Fig. 3 Average inhibition rate of maize germination (a) and root elongation (b) in 72 hr by different TPH contaminated soils.

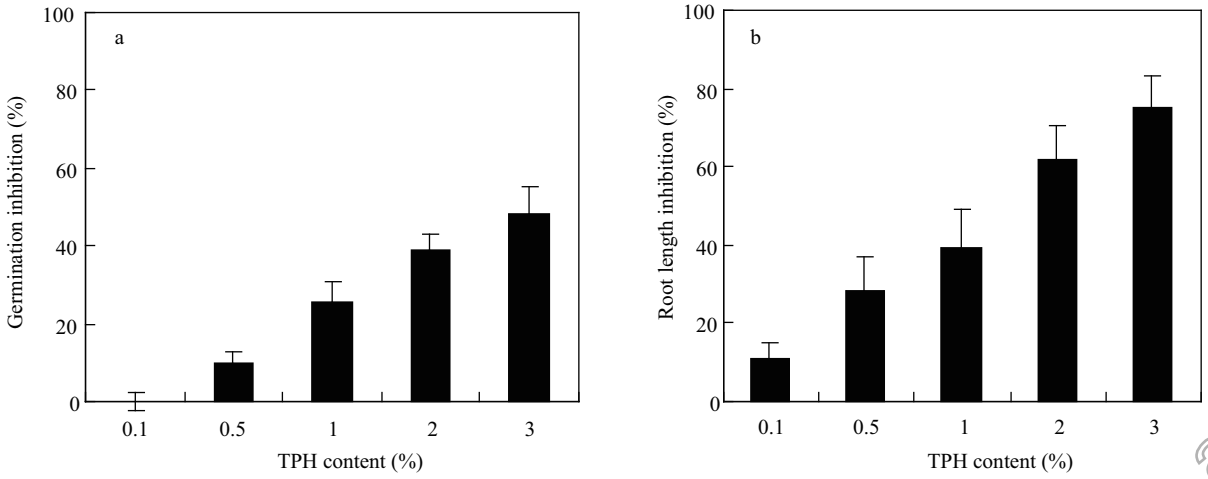

Fig. 4 Average inhibition rate of wheat germination (a) and root elongation (b) in 72 hr by different TPH contaminated soils.

rate increased with increasing TPH content. Significant inhibition was found from TPH content of 2% and the inhibition rate has exceeded 50% at this content level. The EC_{50} for germination and root elongation is 2.86% and 1.64%, respectively. As compared with maize, the wheat is more sensitive in germination and less sensitive in root elongation.

2.3 Luminescent bacteria test

A positive relation was found between relative inhibition rate of luminescent intensity and petroleum concentrations (Fig. 5). EC_{50} value was calculated based on the relation between dilution rate and relatively inhibition rate. The EC_{50} value was 0.47% for the petroleum in the soil. The inhibition rate was nearly 100% when the petroleum content was 1% suggesting high toxicity of petroleum on the bacteria activity.

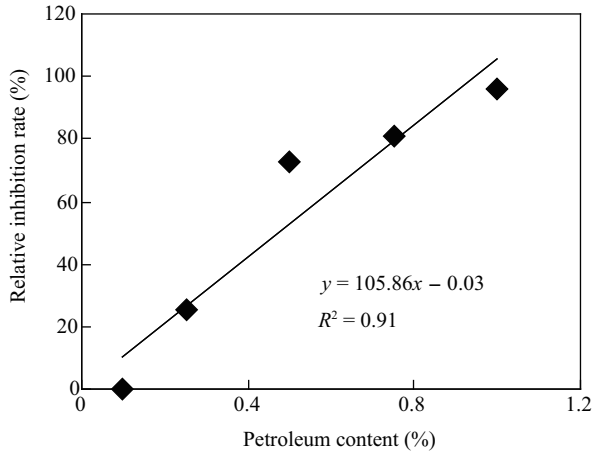

Fig. 5 Toxicity of soil petroleum toxicity by luminescent bacteria method.

3 Discussion

The mortality of *E. fetida* increased in soils contaminated with 2% petroleum (Geissen et al., 2008). The earthworm toxicity test also demonstrated that diesel concentrations in soil exceeding 1% caused a dose-dependent weight loss in earthworms and increased mortality and oil concentration of 1.5% has reduced survival to less than 40% (Hanna and Weaver, 2002; Shin et al., 2005). While another report showed that 0.1% TPH content did not show any lethal effects (Schaefer, 2003). TPH may cause death, swelling, body lesions, stiffening, coiling and low reproduction of earthworm (Oboh et al., 2007). The above result is in accordance with our result with LD_{50} of 1.45% for 4 days experiment in earthworm. The TPH analysis by GC method suggests that C6–C25 hydrocarbons are the more volatile, soluble, and biodegradable constituents in crude oil and may provide an indicator of acute toxicity to worms. Bioavailability and sequestration of TPH as affected by soil properties were considered to be related to the toxicity of petroleum hydrocarbons in the soil (Dorn and Salanitro, 2000). In addition, earthworm is a good option for bioremediation of TPH contaminated soil because it can aerate and bioturbate soils and improve their nutritional status and fertility (Hickman and Reid, 2008; Banu et al., 2005). Earthworm can serve as both indicator of soil toxicity and bioremediation material for petroleum hydrocarbons.

Different plant species may behave differently in evaluating TPH toxicity in the soil with wheat and maize being generally used (Banks and Schultz, 2005; Plaza et al., 2005). Germination studies are considered short-term and primarily assess acute toxicity effects. Six higher plant species (*Secale cereale* L., *Lactuca sativa* L., *Z. mays* L., *Lepidium sativum* L., *Triticum vulgare* L., *Brassica oleracea* L.) were used for bioassay tests based on seed germination and root elongation (Plaza et al., 2005). A higher sensitivity in root elongation was found as compared to germination for both wheat and maize in our study. This is in accordance with Song et al. (2006) in their study the seed germination and seedling growth with the sensitivity of the three parameters in diesel contaminated soil are in descending order: root length > shoot elongation > germination rate. The phytotoxicity may differ depending on different kinds of petroleum. Ogboghodo et al. (2004) reported no germination at 4% for *Escravos* light crude oil pollution. Similarly, all the variables such as germination, growth, aerial, root and total biomass and number of nodules were reduced in contaminated soil as reported by Rivera-Cruz and Trujillo-Narcia (2004). The phytotoxicity of petroleum hydrocarbons may be affected by differences in uptake of oil compounds, nutrient availability, and cell wall structure of different plant species or different plant tissues.

It is suggested that 1.5% is a critical value for both animal toxicity and phytotoxicity based on this study. However, the activity of bacteria can be affected easily by petroleum content of only 0.47%. The sensitivity of the microbial test may be contributed to the use of DCM/DMSO that allows the dispersion of the petroleum hydrocarbons more easily. Shafir et al. (2007) reported that the dispersed oil was significantly more toxic than original crude oil. Bioassays have clearly demonstrated that chemical analysis alone is not adequate to assess the potential ecological impact of contaminated soil and biological tests have been shown to be useful particularly when predicting the effect of a complex mixture of compounds, such as petroleum. Combination of the bioassays with chemical monitoring for evaluating the bioremediation effectiveness and assessing the contaminated/remediated soils is recommended. Sensitivity to petroleum contaminated soils among different biological accepters was concluded as: luminescent bacteria activity > earthworm > plant growth (root elongation and germination).

4 Conclusions

This study demonstrated the ecotoxicity of petroleum pollutants in soil by using earthworm, plant and luminescent bacteria toxicity experiments. The LD_{50} of petroleum contaminated soil for earthworm is 1.45% in 4 days, while

EC_{50} for root elongation was 1.64% and 1.11% for wheat and maize, respectively, and EC_{50} in luminescent bacteria test is about 0.5%. The result suggests much higher toxicity of petroleum on bacteria activity, while some plant species such as wheat is tolerant to petroleum contamination than earthworm and bacteria.

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References

Al-Mutairi N, Bufarsan A, Al-Rukaibi F, 2008. Ecorisk evaluation and treatability potential of soils contaminated with petroleum hydrocarbon-based fuels. *Chemosphere*, 74(1): 142–148.

Banks M K, Schultz K E, 2005. Comparison of plants for germination toxicity tests in petroleum-contaminated soils. *Water Air and Soil Pollution*, 167(1-4): 211–219.

Banu J R, Esakkiraj S, Nagendran R, Logakanthi S, 2005. Biomanagement of petrochemical sludge using an exotic earthworm *Eudrilus eugineae*. *Journal Environmental Biology*, 26(1): 43–47.

Brils J M, Huwer S L, Kater B J, Schout P G, Harmsen J, Delvigne G A L et al., 2002. Oil effect in freshly spiked marine sediment on *Vibrio fischeri*. *Corophium volutator* and *Echinocardium cordatum*. *Environmental Toxicology and Chemistry*, 21(10): 2242–2251.

Chaineau C H, Yepremian C, Vidalie J F, Ducreux J, Ballerini D, 2003. Bioremediation of a crude oil-polluted soil: Biodegradation, leaching and toxicity assessments. *Water, Air, Soil Pollution*, 144(1): 419–440.

Couillard C M, Lee K, Legare B, King T L, 2005. Effect of dispersant on the composition of the water-accommodated fraction of crude oil and its toxicity to larval marine fish. *Environmental Toxicology and Chemistry*, 24(6): 1496–1504.

Di Toro D M, McGrath J A, Stubblefield W A, 2007. Predicting the toxicity of neat and weathered crude oil. Toxic potential and the toxicity of saturated mixtures. *Environmental Toxicology and Chemistry*, 26(1): 24–36.

Dong G H, Chen L J, Wu Z J, Xiao S H, Chen L, Lei E C, 2003. Effect of exogenous selenium on soybean yield and its total N and P contents and on soil enzyme activities. *Chinese Journal of Applied Ecology,* 14(5): 776–780.

Dorn P B, Salanitro J P, 2000. Temporal ecological assessment of oil contaminated soils before and after bioremediation. *Chemosphere,* 40: 419–426.

Geissen V, Gomez-Rivera P, Lwanga E, Mendoza R B, Narcias A T, Marcias E B, 2008. Using earthworms to test the efficiency of remediation of oil-polluted soil in tropical Mexico. *Ecotoxicity Environmental Safety*, 71(3): 638–642.

Hanna S H S, Weaver R W, 2002. Earthworm survival in oil contaminated soil. *Plant and Soil*, 240(1): 127–132.

Hickman Z A, Reid B J, 2008. Earthworm assisted bioremediation of organic contaminants. *Environment International*, 34(7): 1072–1081.

Huang R X, Wen B, Pei Z G, Shan X Q, Zhang S Z, Williams P N, 2009. Accumulation, subcellular distribution and toxicity of copper in earthworm (*Eisenia fetida*) in the presence of ciprofloxacin. *Environmental Science & Technology*, 43(10): 3688–3693.

Jones O A H, Spurgeon D J, Svendsen C, Griffin J L, 2008. A metabolomics based approach to assessing the toxicity of the polyaromatic hydrocarbon pyrene to the earthworm *Lumbricus rubellus*. *Chemosphere,* 71(3): 601–609.

Jonker M T O, Brils J M, Sinke A J C, Murk A J, Koelmans A A, 2006. Weathering and toxicity of marine sediments contaminated with oils and polycyclic aromatic hydrocarbons. *Environmental Toxicology and Chemistry*, 25(5): 1345–1353.

Klok C, Thissen J, 2009. Are laboratory derived toxicity results informative for field situations? Case study on earthworm populations contaminated with heavy metals. *Soil Biology & Biochemistry*, 41(2): 251–255.

Labud V, Garcia C, Hernandez T, 2007. Effect of hydrocarbon pollution on the microbial properties of a sandy and a clay soil. *Chemosphere*, 66(10): 1863–1871.

Lee R F, 2003. Photo-oxidation and photo-toxicity of crude and refined oils. *Spill Science and Technology Bulletin*, 8(2): 157–162.

Li Z P, Liu M, Wu X C, Han F X, Zhang T L, 2010. Effects of long-term chemical fertilization and organic amendments on dynamics of soil organic C and total N in paddy soil derived from barren land in subtropical China. *Soil & Tillage Research,* 106(2): 268–274.

Martinez-Jeronimo F, Villasenor R, Rios G, Espinosa-Chavez F, 2005. Toxicity of the crude oil water-soluble fraction and kaolin-adsorbed crude oil on *Daphnia magna* (Crustacea: Anomopoda). *Archives of Environmental Contamination and Toxicology*, 48(4): 444–449.

Neff J M, Ostazeski S, Gardiner W, Stejskal I, 2000. Effects of weathering on the toxicity of three offshore Australian crude oils and a diesel fuel to marine animals. *Environmental Toxicology and Chemistry*, 19(7): 1809–1821.

Oboh B O, Adeyinka Y, Awonuga S, Akinola M O, 2007. Impact of soil types and petroleum effluents on the earthworm, *Eudrilus eugeniae*. *Journal of Environmental Biology*, 28(2): 209–212.

OECD (Organization for Economic Cooperation and Development), 2000. Proposal for Updating Guideline 208: Terrestrial (Non-Target) Plant Test 208A–Seedling emergence and seedling growth test. European Committee, Paris, France. 208–209.

Ogboghodo I A, Iruaga E K, Osemwota I O, Chokor J U, 2004. An assessment of the effects of crude oil pollution on soil properties, germination and growth of maize (*Zea mays*) using two crude types – Forcados light and Escravos light. *Environmental Monitoring and Assessment*, 96(1-3): 143–152.

Owojori O J, Reinecke A J, Rozanov A B, 2008. Effects of salinity on partitioning, uptake and toxicity of zinc in the earthworm *Eisenia fetida*. *Soil Biology & Biochemistry*, 40(9): 2385–2393.

Perkins R A, Rhoton S, Behr-Andres C, 2005. Comparative marine toxicity testing: A cold-water species and standard warm-water test species exposed to crude oil and dispersant. *Cold Regions Science and Technology*, 42(3): 226–236.

Plaza G, Nalecz-Jawecki G, Ulfig K, Brigmon R L, 2005. The application of bioassays as indicators of petroleum-contaminated soil remediation. *Chemosphere*, 59(2): 289–296.

Rivera-Cruz D C M, Trujillo-Narcia A, 2004. Study of plant toxicity in soils with new and tempered petroleum. *Interciencia,* 29(7): 369–376.

Schaefer M, 2003. Behavioural endpoints in earthworm ecotoxicology: Evaluation of different test systems in soil toxicity assessment. *Journal of Soils and Sediments,* 3(2): 79–84.

Shafir S, Rijn J V, Rinkevich B, 2007. Short and long term toxicity of crude oil and oil dispersants to two representative coral species. *Environmental Science & Technology,* 41: 5571–5574.

Shin K H, Jung H Y, Chang P C, Choi H C, Kim K W, 2005. Earthworm toxicity during chemical oxidation of diesel-contaminated sand. *Environmental Toxicology and Chemistry*, 24(8): 1924–1929.

Song X Y, Song Y F, Sun T H, Li X X, Zhang W, Zhou Q X, 2006. Soil-based eco-toxicity of diesel oil contamination to seed germination and seedling growth of wheat. *Journal of Agro-Environment Science,* 25(3): 554–559.