IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)
ISSN (P): 2347-4580; ISSN (E): 2321-8851
Vol. 7, Issue 5, May 2019, 1-16

ENZYMES ACTIVITIES AS BIO INDICATOR OF SOIL CONTAMINATED WITH PETROLEUM HYDROCARBON

Nyoyoko, Veronica Fabian[1], Anyanwu Chukwudi U[2] & Christorpher, Mary Anthony[3]

[1,2]Research Scholar, Department of Microbiology, University of Nigeria, Nsukka, Nigeria

[3]Research Scholar, Department of Microbiology, AkwaIbom State University, Mkpat-Enin, Nigeria

Received: 25 Mar 2019 **Accepted:** 04 May 2019 **Published:** 14 May 2019

ABSTRACT

The study evaluates the changes in the soil dehydrogenase and urease contaminated with hydrocarbon. Soil dehydrogenase and urease activity were investigated by contaminating soils at five loading rates (1.0, 5.0, 10, 15, 20 %) volume of oil/weight of soil and monitoring activity at 7 days interval. The highest level of the dehydrogenase activity observed at 21 days of incubation in crude oil contaminated soil was 1.19 ± 0.01 and at 7 days in kerosene contaminated soil was 0.78 ± 0.01. The highest level of the urease activity observed at 21 days of incubation in crude oil contaminated soil was 1.34 ± 0.01 and at 7 days in kerosene contaminated soil was 1.25 ± 0.01. The increase in dehydrogenase and urease activity was in proportion to the rate of oil application, as it increases with increasing loading rates. Analysis of variance of dehydrogenase activity and urease activity showed a high significant difference between the control and the oil treated soils at $p < 0.05$ level. The study demonstrated that soil contaminated with crude oil and kerosene disturbed the biochemical equilibrium of soil. Dehydrogenase and urease activity may be employed as suitable tools for predicting, assessing and remediating effect of crude oil and kerosene on the soil.

KEYWORDS: *Dehydrogenase, Urease, Crude Oil, Kerosene, Bioindicator, Pollution*

INTRODUCTION

Despite the several usefulness of crude oil and its products, it also constitutes major environmental concern, globally (Hentati*et al.*, 2013; Saratale*et al.*, 2007). Environmental Pollution caused by crude and refined oils is a threat to the environment and it inhabitants (Xu*et al.*, 2018). Assessment and remediation of the effects are major challenges to environmental researchers. The occurrence of oil spillage mostly put farmers at a disadvantaged position because changes in the chemical and biological properties of the soil either affect yield positively or negatively (Ikpeme*et al.*, 2007; Kalme*et al.*, 2000). The effect of this pollution varies due to the difficulty in assessing it due to limited knowledge of the additive, synergistic or antagonistic effects of mixtures involved (Saterbaca*et al.*, 2000,). The chemical composition of crude oil and kerosene varies significantly and can have diverse effects on soil(Hou*et al.*,2018). There is a need to have suitable tools for predicting, assessing and remediating effect of crude oil and kerosene on soil (Guerra *et al.*,2018).

A bioindicator is an organism, part of an organism, the product of an organism (enzyme), collection of organisms or biological process which can be used to obtain information on the quality of all or part of the environment (Killham, 2002 ; Zornoza*et al.*, 2015). Bioindicators are very important for resource managers in order to understand ecological changes within the soil ecosystem (Dale *et al.*, 2008). Soil biological activity, including enzymatic activity, is influenced

by a range of physiochemical, environmental parameters and perturbations. Therefore, soil enzymatic activity may be used to assess disturbed soil (Labud *et al.*, 2007). Soil enzymes are a group of enzymes found in the soil and are continuously playing important roles in maintaining soil ecology, physical and chemical properties, fertility, and soil health. These enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system (Zahir *et al.*, 2001; Tejada, 2009). Alteration or toxicity of soil enzymes activity affect soil quality (Lopes, 2011), soil fertility (Abii and Nwosu, 2009), lead to ecological stress (Tejada, 2009), increase in organic carbon of the soil with a concomitant decrease in soil nitrate (Okolo*et al.*, 2005). Soil enzymes have been used to assess soil quality and healthas affected by agricultural practices (Gianfreda*et al.*, 2005; Truu *et al.*, 2008; Garcia-Ruiz *et al.*, 2009) and as potential soil quality indicators because they are sensitive to ecological stress and land management practices (Tejada, 2009). It has been shown that enzymes react to changes in soil management more quickly than other variables and therefore may be useful as early indicators of biological changes (Zabaloy*et al.*, 2008). The use of soil enzyme in the evaluation of soil quality becomes a more valuable tool since culturable and unculturable, even extra-cellular and intracellular enzyme activities are estimated in the process. The enzymes that can be investigated in polluted soils include dehydrogenase, urease, polyphenol oxidase, hydrogen peroxidase, acid and alkaline phosphatase (Oliveira and Pampulha, 2006; Li *et al.*, 2005; Nwaugo*et al.*, 2008).

Dehydrogenases are enzymes which catalyze the removal of the hydrogen atom from different metabolites (Nelson and Cox, 2000). Active dehydrogenases are considered to exist in the soil as an integral part of intact cells and conduct a broad range of oxidative activities that are responsible for the degradation of soil organic matter (Margesin*et al.*, 1997). Dehydrogenase activity (DHA) has been proposed as a sensitive indicator for evaluating microbial oxidative activity in soils (Turgay*et al.*, 2010; Serrano *et al.*, 2009; Dawson *et al.*, 2007). It can also indicate the type and significance of pollutant on the microbiological quality of soils (Xie*et al.*, 2009; Tejada*et al.*, 2010). Compounds such as soluble tetrazolium salts are reduced to red colored formazans, which can be extracted and measured colorimetrically (Tabatabai*et al.*, 1997; Shaw and Burns, 2006). Soil dehydrogenase activity reflects changes in the respiratory activity of given population size in response to changes in the soil environment (Schinner*et al.*, 1996).

In evaluating changes in soil quality as it relates to management, the enzyme urease has been widely used (Saviozzi*et al.*, 2001). This enzyme, in most cases, is an extra-cellular enzyme representing up to 63% of total activity in the soil (Martinez-Salgado *et al.*, 2010). It has been shown that urease activity depends on the microbial community, physical, and chemical properties of soil (Corstanje*et al.*, 2007). Dehydrogenases, catalases, and urease have been found to be useful for indicating the onset of the biodegradation process, as their activities decline rapidly after the rate of biodegradation have decreased (Margesin and Schinner, 1997). The increase in soil dehydrogenase and urease activity in hydrocarbon contaminated soil has been found to be in proportion to the rates of oil application, in which activity increased with increasing loading rates. In addition, any influence that oils may have on soil dehydrogenase and urease activity is dependent on chemical composition. The objective of this study was to investigate dehydrogenase and urease activity in soil contaminated with crude oil and kerosene.

MATERIALS AND METHODS

Bonny light crude oil was collected from Exxon Mobil, Eket in Akwa Ibom State. Soil samples were randomly collected with the aid of auger from the University of Nigeria, Nsukka agricultural farmland. The soil samples collected were bulked, air dried and sieved to remove coarse fragments. Soil sample (100 g) was weighed into a conical flask and

amended with crude oil and kerosene oil (0%, 1%, 5%, 10%, 15% and 20%, volume per weight), respectively. The oil was thoroughly mixed with the soil in the conical flask. Soil sample amended with crude oil (0%, 1%, 5%, 10%, 15%, and 20 %, v/w) and kerosene oil (0%, 1.0%, 5.0%, 10%, 15%, and 20 %, v/w), in conical flasks were plugged with cotton wool**.** Each set up was arranged in triplicate, incubated at 28^0C, analyzed at 0, 7, 14, 21 and 28 days respectively for enzyme activity.

Analysis of Soil Enzymes Activities

The activities of enzymes dehydrogenase and urease were assessed by evaluating the dehydrogenase and urease activity.

Evaluation of Dehydrogenase Activity in the Soil

Dehydrogenase activity was determined using the method described Casida (1977) modify by Tabatabai (1997).

A 0.5-gram portion of the soil was placed in a test tube (15x100 mm) and mixed with 0.5 ml of 3 % (w/v) aqueous 2, 3,5 –triphenyl tetrazolium chloride (TTC), stirred with a glass rod and incubated. After 24 hours of incubation, 5 ml of ethanol was added to the test tube and the suspension was vortexed for 30 seconds. The tube was incubated for 1 hour to allow suspended soil to settle. The resulting supernatant (5ml) was carefully transferred to the clean test tube using Pasteur pipette. Absorbance was read spectrophotometrically at 485 nm. The unit of dehydrogenase activity was reported as mg triphenyltetrazoliumformazan (TPF) released/g soil per 24 hrs (Ohlinger, 1996).

Evaluation of Urease Activity in the Soil

Urease activity was measured as described by Tabatabai (1997). A 0.5gram portion of the soil was placed in a test tube, with 0.5 ml of 10%, urea substrate solution added subsequently and mixed thoroughly. The contents were allowed to stand for approximately 24 hrs. After 24 hrs of incubation, 4.5 ml of saturated calcium sulphate ($CaSO_4$) solution was added, shaken for 30 minutes and allowed to settle for 10 minutes. Three ml of the supernatant was transferred into another test tube and 2 ml of the color reagent (a mixture of p-dimethylamino-benzaldehyde (2.0g), 95% ethyl alcohol (100 mL) and concentrated HCl (10 ml)) added, mixed and allowed to stand for 10 minutes. The absorbance was read at 420 nm**.** The unit of urease activity was reported as mg Urease (NH^4+-N) released/g of soil / 24 hrs.

RESULTS AND DISCUSSIONS

Physicochemical Properties of Soil

The results of physicochemical properties of the soil sample determined are presented on Table 1. The soil was identified and classed as sandy loam. The pH of the soil was acidic: 4.55 ± 0.49; moisture content: 23.97%; organic carbon: 0.99% ; organic matter: 1.17% ; nitrogen: 0.098%; clay and silt: 32%; fine sand: 36%; coarse sand: 40%; saturated base: 32.82%; Phosphurus: 31.71 ppm; cation exchange capacity: 14.80 (meq/100 g); exchangeable acid: 2.80 (meq/100 g) and exchangeable base meq /100 g; sodium, 0.028; potassium: 0.230; calcium: 2.80 and magnesium: 1.80 (meq /100 g).

Table 1: Physiochemical Properties of Soil Sample

Parameter	Values(0- 15cm depth
Texture class	Sandy loamy
Particle size (Clay & Silt)	32 %
Particle size (Fine sand)	36 %
Coarse sand	40 %
pH value	4.55±0.49
Moisture content	23.97 %;
Carbon	0.99 %
Organic matter	1.72 %
Nitrogen	0.098 %
Exchangeable bases: Sodium	0.028 (meq /100 g)
Potassium	0.230 (meq /100 g)
Calcium	2.80 (meq /100 g)
Magnesium	1.80 (meq /100 g)
Cation exchange capacity	14.80 (meq /100 g)
Saturated base	32.82 %
Exchangeable acidity	2.80 (meq/100 g)
Phosporus	31.71 ppm

Effect of Crude Oil on Soil Dehydrogenase Activity

The concentration of crude oil on the activity of soil dehydrogenase is shown in Figure 1. The dehydrogenase activity at 0 % crude oil treatment ranged from 0.35 ± 0.01 to 0.36 ± 0.01; 0.35 ± 0.01 to 0.59 ± 0.01 and decrease to 0.44 ± 0.01 for 1.0 %; 0.35 ± 0.00 to 0.76± 0.01 and decrease to 0.49 ± 0.01 for 5 %; 0.36 ± 0.00 to 0.92 ± 0.01 and decrease to 0.56± 0.01 for 10%; 0.36 ± 0.00 to 1.05 ± 0.011 and decrease to 0.62 ± 0.01 for 15% and 0.37± 0.01 to1.19 ± 0.01 and decrease to 0.75 ± 0.02 for 20 %.

Effect of Kerosene on Soil Dehydrogenase Activity

Figure 2 shows the dehydrogenase activity in the soil treated with various concentrations of kerosene. The dehydrogenase activity at 0 % kerosene treatment ranged from 0.35 ± 0.00 to 0.38 ± 0.01 decrease to 0.37 ± 0.01; 0.35 ± 0.01 to 0.42 ± 0.01 and decrease to 0.39 ± 0.01 for 1.0 %; 0.35 ± 0.01 to 0.49 ± 0.01 and decrease to 0.41 ± 0.01 for 5 %; 0.35 ± 0.00 to 0.57 ± 0.01 and decrease to 0.47 ± 0.01 for 10%; 0.35 ± 0.01 to 0.63 ± 0.02 and decrease to 0.48 ± 0.01 for 15% and 0.36± 0.00 to0.78 ± 0.01 and decrease to 0.54 ± 0.00 for 20 %.The comparative effects of both crude oil and kerosene on soil dehydrogenase activity are shown in Table 2.

Effect of Crude Oil on Soil Urease Activity

The concentration of crude oil on the activity of soil urease is shown in Figure 3. The urease activity at 0 % crude oil treatment ranged from 1.14 ± 0.00 to 1.14 ± 0.00; 1.16 ± 0.01 to 1.22 ± 0.01 and decrease to 1.21 ± 0.01 for 1.0 %; 1.16 ± 0.01 to 1.25 ± 0.01 and decrease to 1.22 ± 0.01 for 5 %; 1.16 ± 0.30 to 1.28 ± 0.01 and decrease 1.27 ± 0.01 for 10%; 1.17 ± 0.01 to 1.30± 0.01 and decrease to 1.28 ± 0.01 for 15% and 1.18 ± 0.00 to1.34 ± 0.013 and decrease to 1.32 ± 0.01 for 20 %.

Effect of Kerosene on Soil Urease Activity

The concentration of kerosene on the activity of soil urease is shown in Figure 4. The urease activity at 0 % kerosene treatment ranged from 1.14 ± 0.01 to 1.16 ± 0.01 and decrease to 1.14 ± 0.01 ; 1.15 ± 0.01 to 1.17 ± 0.01 and decrease to 1.15 ± 0.01 for 1.0 %; 1.15 ± 0.01 to 1.18 ± 0.01 and decrease to 1.15 ± 0.01 for 5 %; 1.16 ± 0.01 to 1.20 ±

0.00 and decrease to 1.16 ± 0.01 for 10%; 1.16 ± 0.006 to 1.22 ± 0.01 and decrease to 1.17 ± 0.01 for 15% ; 1.17 ± 0.00 to1.25 ± 0.01 and decrease to 1.18 ± 0.01 for 20 %.The comparative effects of both crude oil and kerosene on soil urease activity are shown in Table 3.

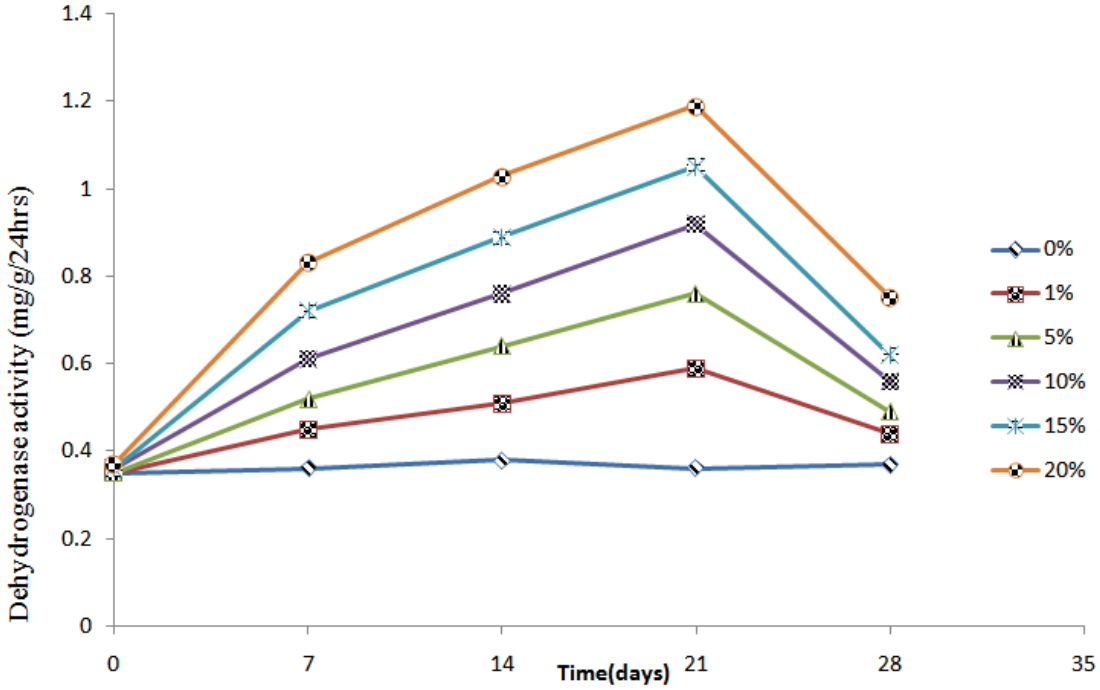

Figure 1: Effect of Crude Oil on Dehydrogenase Activity in the Soil

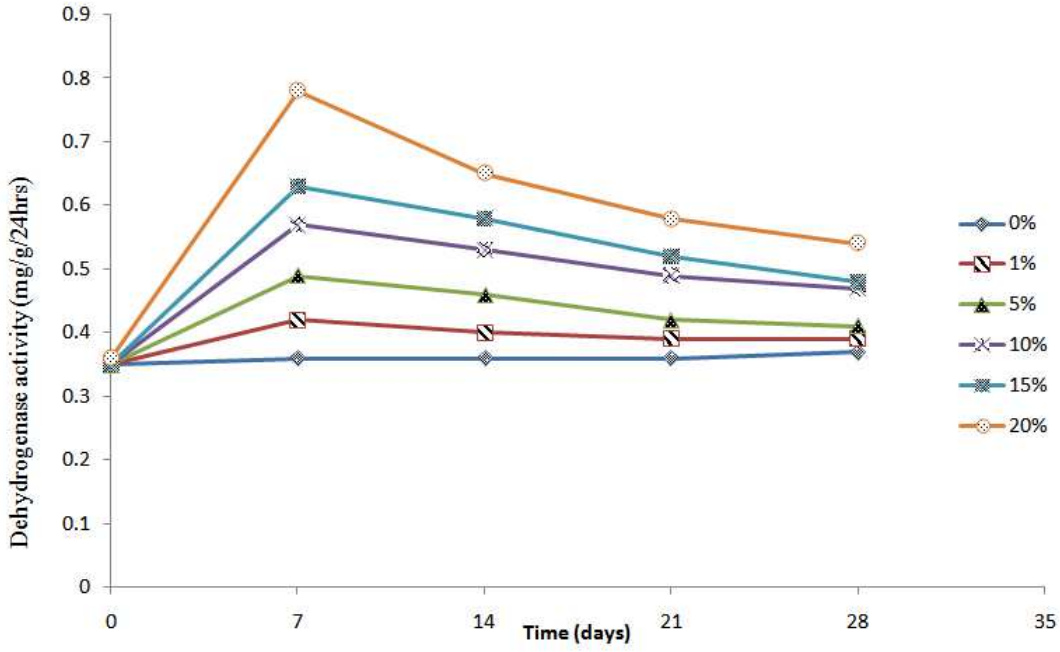

Figure 2: Effect of Kerosene on Dehydrogenase Activity in the Soil

Table 2: Dehydrogenase Activity in Soil Contaminated with Crude Oil and Kerosene (mg/g/24hrs)

Day	0.0%	1.0%	5.0%	10.0%	15.0%	20%
0 Cr	0.35 ±0.01	0.35 ± 0.01	0.35 ± 0.00	0.36 ± 0.00	0.36 ± 0.00	0.37± 0.00
Kr	0.35 ±0.00	0.35 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.36 ± 0.00
7 Cr	0.35 ± 0.01	0.45 ± 0.01	0.52 ± 0.00	0.61 ± 0.00	0.72 ± 0.05	0.83 ± 0.00
Kr	0.36 ± 0.00	0.42 ± 0.00	0.49 ± 0.01	0.57 ± 0.00	0.63 ± 0.01	0.78 ± 0.00
14 Cr	0.36 ± 0.00	0.51 ± 0.01	0.64 ± 0.00	0.76 ± 0.00	0.89 ± 0.00	1.03 ±0.02
Kr	0.38 ± 0.01	0.40 ± 0.00	0.46 ± 0.01	0.53 ± 0.00	0.58 ± 0.00	0.65±0.00
21 Cr	0.36 ± 0.00	0.59± 0.00	0.76± 0.01	0.92 ± 0.01	1.05 ± 0.01	1.19± 0.00
Kr	0.36 ± 0.00	0.39 ± 0.01	0.42± 0.00	0.49 ± 0.01	0.52 ± 0.03	0.58 ± 0.02
28 Cr	0.36 ± 0.00	0.44± 0.00	0.49 ± 0.00	0.56± 0.01	0.62 ± 0.00	0.75 ± 0.01
Kr	0.37 ± 0.00	0.39 ± 0.01	0.41± 0.01	0.47 ± 0.00	0.48 ± 0.01	0.54± 0.01

Key: Cr crude oil
Kr: kerosene

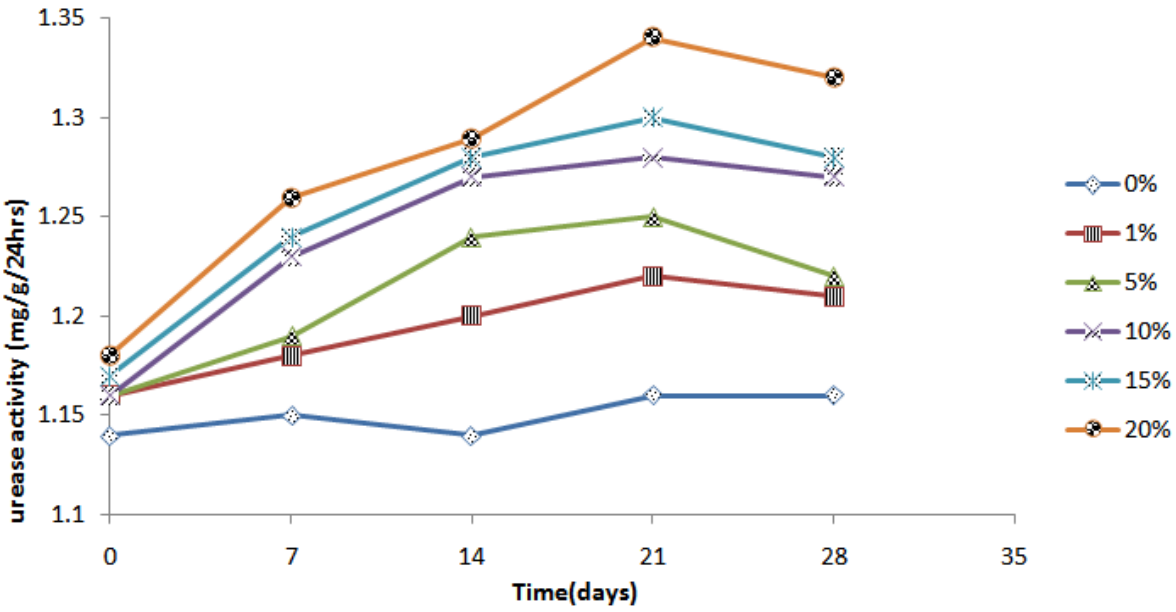

Figure 3: Effect of Crude Oil Contaminated Soil on Urease Activity in the Soil

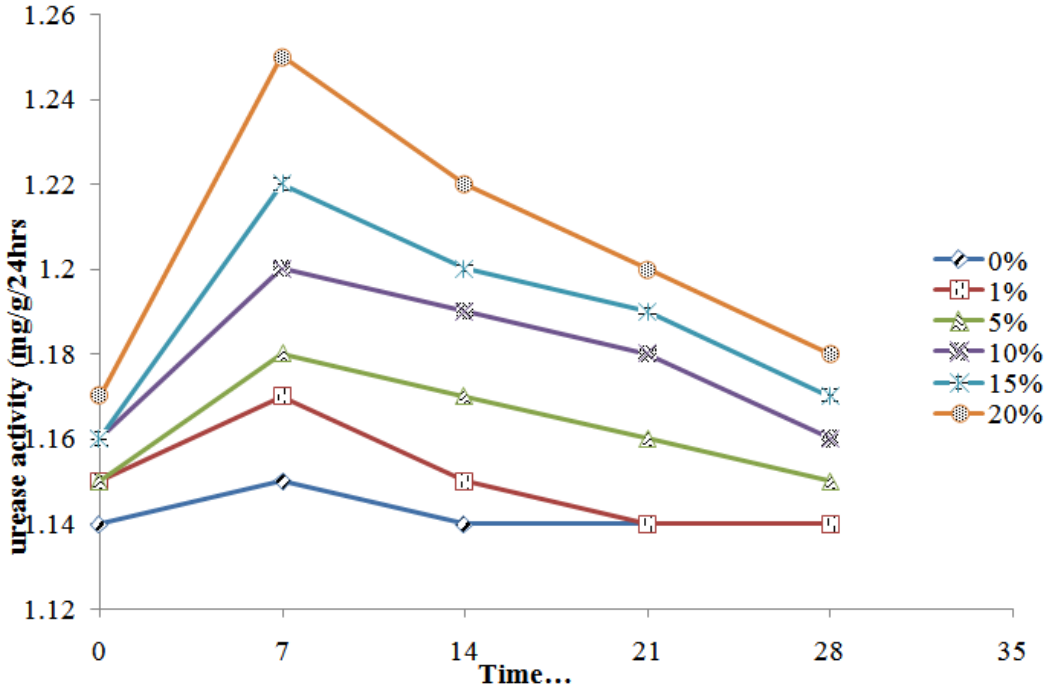

Figure 4: Effect of Kerosene Contaminated on Urease Activity in the Soil

Table 3: Urease Activity on Soil Contaminated with Crude Oil and Kerosene (mg/g/24hrs)

Day	0.0%	1.0%	5.0%	10.0%	15.0%	20%
0 Cr	1.14 ± 0.00	1.16 ± 0.00	1.16± 0.01	1.16 ± 0.00	1.17 ± 0.00	1.18 ± 0.00
Kr	1.14 ±0.00	1.15 ±0.00	1.15± 0.00	1.16 ±0.01	1.16 ±0.01	1.17 ± 0.00
7 Cr	1.15 ± 0.00	1.18 ± 0.01	1.19 ± 0.00	1.23 ± 0.01	1.24 ± 0.01	1.26 ± 0.00
Kr	1.15 ±0.00	1.17 ±0.00	1.18 ±0.01	1.20 ±0.00	1.22 ±0.01	1.25 ± 0.00
14 Cr	1.14± 0.00	1.20 ± 0.01	1.24 ± 0.02	1.27 ± 0.01	1.28 ± 0.01	1.29 ± 0.01
Kr	1.16 ±0.00	1.15 ±0.01	1.17 ±0.00	1.19± 0.01	1.20 ±0.00	1.22± 0.00
21 Cr	1.14 ± 0.01	1.22 ± 0.00	1.25 ± 0.04	1.28 ± 0.00	1.30± 0.01	1.34 ± 0.01
Kr	1.14 ±0.00	1.14 ±0.01	1.16 ±0.00	1.18± 0.01	1.19 ±0.00	1.20± 0.00
28 Cr	1.14 ± 0.00	1.21 ± 0.00	1.22 ± 0.02	1.27 ± 0.011	1.28 ± 0.013	1.32 ± 0.006
Kr	1.14 ±0.00	1.14 ±0.01	1.15 ±0.01	1.16 ±0.003	1.17 ±0.006	1.18 ± 0.001

Key: Cr crude oil
Kr: kerosene

The highest level of dehydrogenase activity observed at 21 days of incubation in crude oil contaminated soil was 1.19 ± 0.01 (mg/g/24hrs), while it was at 7 days in kerosene contaminated soil it was 0.78 ± 0.01 (mg/g/24hrs). The highest level of the urease activity observed at 21 days of incubation in crude oil contaminated soil was 1.34 ± 0.01 (mg/g/24hrs) and at 7 days in kerosene contaminated soil, it was observed to be 1.25 ± 0.01 (mg/g/24hrs).

The statistical analysis of dehydrogenase and urease activity in the control and crude oil-polluted soils showed that there was a significant difference ($P<0.05$) at the oil contaminated soils (1%, 5%, 10%, 15%, and 20%) in the enzymes activity and the control. The statistical analysis of dehydrogenase and urease activity in the control and kerosene-polluted soils showed that there was a significant difference ($P<0.05$) at the oil contaminated soils (1%, 5%, 10%, 15%, and 20%) in the enzymes activity and the control. The order of decreasing average concentration of dehydrogenase and urease in the soils treated with various concentrations of crude oil and kerosene was 20 % > 15% > 10% > 5 % > 1 % > 0 %

From the dehydrogenase and urease, it was found that the crude oil could destabilize the dehydrogenase and urease activity, by a rapid increase from 7 days to 21days and decreases after 21 days. Kerosene could also destabilize the dehydrogenase and urease at initial phase followed by a rapid increase in 7 days and decreases after 14 days. After 24 hours of pollution, the concentration of crude oil had a significant effect on the activity of soil dehydrogenase and urease. The result also revealed that soil enzyme activity at higher levels of pollution was significantly higher compared to the control and the 1% crude oil treatment. The soil dehydrogenase and urease activities from the sample polluted with the crude oil of higher concentration were higher than those polluted by lower concentration. This is due to some organic material in petroleum which could increase the concentration of hydrogen ion, which led to an increase in the value of dehydrogenase and urease activity.

The contamination of crude oil and kerosene tends to make the soil enzymes unstable and the different effects of hydrocarbons on soil enzyme activity were depending on the composition of the crude oil and kerosene, and the quantity reaching the soil, which agree with the results of Andreson and Khaziev (1981); Kaigorodova (1996); Zimenka and Kartyzhova (1986); Serrano, (2009); Kiss (1998), Li, (2005); Arezo and Vanid, (2013) in their studies.

The level of dehydrogenase activity was dependent on the amount and type of oil added. This corresponds with Mikkonen*et al.* (2011) who reported that the stimulation of dehydrogenase and urease activity on petroleum hydrocarbon contaminated soil occurs immediately after the contamination event and then decreases gradually over time. Margesin*et al.* (2000) and Ueno,(2007) also reported that the addition of petroleum hydrocarbon to soil causes an increase in all enzyme activities as a result of the development of heterotrophic microorganisms in contaminated soil(Chen *et al.*,2017; Mukherjee *et al.*, 2017; Song *et al.*, 2017. The increase in soil enzymatic activities suggests the availability of the high quantity of biodegradable substrates (Bacosa*et al.*,2018; Wanapaisan *et al.*,2018; Tejada*et al*., 2007).

The presence of petroleum aromatic hydrocarbons (PAHs) was said to destabilize the activity of dehydrogenase and most hydrolases. More specifically, the microorganisms involved in C, N, P and S cycling were almost totally decimated, that is, the metabolic capacity of the soil was almost totally paralyzed(Hou*et al.*,2018). The decrease in soil enzymatic activity in response to contamination may also be caused by non-polar organic compounds covering both organic-mineral and cell surfaces, thus hindering the interaction between enzyme active sites and soluble substrates, with adverse effects on the expression of enzyme activity (Kiss, 1998; Andreoni, 2004; Wang *et al*.,2018; Zhao *et al.*,2017). It was observed that the enzyme activity (dehydrogenase and urease) reached similar values with those measured in uncontaminated soils (Serrano, 2009). This suggests that the contaminants undergo some type of transformation that reduces the degree of toxicity to the soil.

Contamination of soil is a particularly serious problem because of the impact that it has on soil functioning, and on the whole ecosystem. Agricultural soils, which are continually exploited to produce food and fodder, are particularly

sensitive to contamination as agricultural soils generally display poor resilience, that is they are incapable of recovering from any type of aggression, and any type of contamination, The effect of crude oil and kerosene brought about alterations to soil functioning.

CONCLUSIONS

The results of this study show that increased in the concentration of crude oil and kerosene, increases the activity of enzymes and thus alters the biochemical functions in the soil system and affects the soil quality, soil stability, soil property, microbial activities, and agricultural production. It may be a good option for study of contaminated soil.

ACKNOWLEDGEMENTS

The authors are grateful to the Microbiology Unit, Department of Biological Sciences, University of Nigeria, Nsukka, for providing us with the laboratory materials and facilities used in this investigation and we also appreciate Rev. Fr. Prof. Vincent Nyoyoko, for funding the research.

REFERENCES

1. *Abii, T. A. and Nwosu, P. C. (2009).The effect of oil-spillage on the soil of Eleme in Rivers State of the Niger Delta area of Nigeria.Research Journal of Environmental Sciences, 3(3): 316-320.*
2. *Achal, V., Kumari, D. and Pan, X. (2011). Bioremediation of chromium contaminated soil by brown-rot fungus, Gloeophyllumsepiarium. Researh in Microbiology, 6: 166- 171.*
3. *Andreoni, V., Cavalca, L., Rao, M., Nocerino, G. and Bernasconi, S. (2004). Bacterial communities and enzymatic activities in polycyclic aromatic hydrocarbons (PAHs) polluted soils. Chemosphere 57:401-412*
4. *Andreson R.K and Khaziev F.K. (1981).Fighting against petroleum pollution of soils.KorroziyaiZashchita v NeftegazovoiPromyshlennosti (Moscow). Russian, 7: 1-46*
5. *Anon. (2013a).Definition and meaning of crude oil.Retrieved June 20, 2013.Available in www.businessdictionary.com/definition/.*
6. *Anon. (2013b).Glossary of Soil Science Terms. Soil Science Society of America.Retrieved May 10, 2012. Available in http://en.m.wikipedia.org/wiki/.*
7. *Arezo, M. B. and Moghanlo, V. S. (2013). Evaluation the activity of some Enzymes and Number of hetrotrophic and degrading bacteria in crude-oil contaminated soil. International Journal of Agronomy and Plant Production. 4 (6): 1134-1142.*
8. *Bacosa, H. P., Erdner, D. L., Rosenheim, B. E., Shetty, P., Seitz, K. W., Baker, B. J., et al. (2018). Hydrocarbon degradation and response of seafloor sediment bacterial community in the northern Gulf of Mexico to light Louisiana sweet crude oil.ISME J. 12, 2532–2543.doi: 10.1038/s41396-018-01901*
9. *Bento, F.M., Camargo, F.A.D.O., Okeke, B.C. and Frankenberger, W.T. (2005). Diversity of biosurfactant*

producing microorganisms isolated from soils contaminated with diesel oil. Microbiology Research, 160: 249-255.

10. *Black, C.A. (2000). Method of Soil Analysis II.American Society of Agronomy, Madison.573-590.*

11. *Bremner, J.M. (1965).Total Nitrogen In: Black, C.A., Evans D.D., Whils J.L., Ensminger L.E. and Clerk F.E. (Eds.), Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. American Society of Agronomy Inc. Madison, Wisconsin, Agronomy, 9: 1149-1178.*
12. *Casida,.L. E.JR. (1977). Microbial Metabolic Activity in Soil as Measured by Dehydrogenase Determinations, Applied And Environmental Microbiology, p. 630-636.*
13. *Chaudhry, S., Luhach, J., Sharma, V. and Sharma, C. (2012). Assessment of diesel degrading potential of fungal isolates from sludge contaminated soil of petroleum refinery, Haryana. Research Journal of Microbiology, 7: 182-190.*
14. *Cheesbrough, M. (2006).District Laboratory Practice in Tropical Countries, Part 2.Cambridge University Press, United Kingdom. 62-70*
15. *Chen, F. S., Zeng, D. H., Fahey, T. J. and Liao, P.F. (2010).Organic carbon in soil physical fractions under different-aged plantations of Mongolian pine in semi-arid region of Northeast China.Applied Soil Ecology. 44:42–48.*
16. *Chen, W., Li, J., Sun, X., Min, J., and Hu, X. (2017).High efficiency degradation of alkanes and crude oil by a salt-tolerant bacterium Dietzia species CN-3.Int. Biodeterior. Biodegrad.118, 110–118. doi: 10.1016/j.ibiod.2017.01.029*
17. *Corstanje, R., Schulin, R. and Lark, R. (2007). Scale - dependent relationships between soil organic matter and urease activity. - European Journal of Soil Science, 58 (5): 1087-95.*
18. *Dawson, J.J.C., Godsiffe, E.J., Thompson, I. P., Ralebitso-Senior, T.K., Killham, K.S. and Paton, G.I. (2007). Application of biological indicators to assess recovery of hydrocarbon-impacted soils.Soil Biology and Biochemistry, 39:164–177.*
19. *Ekpo, M. A. and Ekpo, E. I. (2006). Utilization of Bonny light and Bonny medium crude oils by microorganism isolated from Qua Ibo River Estuary. Nigerian Journal of Microbiology, 20: 832-839.*
20. *Garcia-Ruiz,.R., Ochoa, V., Vinegla, B., Hinojosa, M. B., Pena-Santiago, R., Liébanas, G., Linares, J. C. and Carreira, J. A. (2009). Soil enzymes, nematode community and selected physico-chemical properties as soil quality indicators in organic and conventional olive oil farming: influence of seasonality and site features. Applied Soil Ecology, 41: 305–314.*
21. *Gee, G.W. and Bauder, J.W. (1986). Particle-size analysis.In A. Klute (Editor) Methods of Soil Analysis.Part 1.2nd edition.Agronomy.Monograph. 9. American Society of Agronomy and Soil Science Society of America. p.*

383–411.

22. *Gianfreda, L., Rao, M.A., Piotrowska, A., Palombo, G. and Colombo, C. (2005). Soil enzyme activities as affected by anthropogenic alterations: Intensive agricultural practices and organic pollution. Science of the Total Environment, 341: 265–279.*
23. *Gramss, G., Gunther, T. H. and Fritsche, W. (1998). Spot tests for oxidative enzymes in ectomycorrhizal, wood- and litter decaying fungi. Mycological Research, 102: 67-72.*
24. *Guerra, A. B., Oliveira, J. S., Silva-Portela, R. C., Araujo, W., Carlos, A. C., Vasconcelos, A. T. R., et al. (2018). Metagenome enrichment approach used for selection of oil-degrading bacteria consortia for drill cutting residue bioremediation. Environmental Pollution.235, 869–880.*
25. *Hentati, O. R., Lachhab, R., Ayadi, M. and Ksibi, M. (2013). Toxicity assessment for petroleum-contaminated soil using terrestrial invertebrates and plant bioassays, Environmental Monitoring and Assessessment, 185: 2989-2998.*
26. *Hofman, J., Svihalek, J. and Holoubek, I. (2004).Evaluation of functional diversity of soil microbial communities - a case study.Plant, Soil and Environment, 50: 141-148.*
27. *Holt, J. G., Krieg, N. R., Sneath, P. H. A. and Williams, S. T. (1994).Bergey's Manual of Determinative Bacteriology, 9th Edition, Lippincott, Williams and Wilkins: Baltimore.*
28. *Ikpeme, E.M., Nfongeh, J. F. and Etim, L. (2007).Comparative bioremediation enhancement procedures on kerosene-polluted ultisol from Niger Delta region, Southern Nigeria.Research Journal of Microbiology. 2(11): 856-860.*
29. *Jackson, M. L. (1970).Soil Chemical Analysis New Jersey, Prentice Hall Inc. p 498.*
30. *Kaigorodova S (1996). Biological activity of the petroleum-polluted bog soils in the Tyumen region. Programma in Tezisy 4.NauchnoiKonferentsiiInstitutaBiologii 'Aktual'nyeProblemyBiologii, Syktyvkar in Russian.*
31. *Kalme, S., Parshetti, G., Gomare, S. and Govindwar, S. (2008).Diesel and kerosene degradation by P. desmoyticumNCIM 2112 and Nocardia hydrocarbon oxydansNCIM 2386.Current Microbiology, 56:581- 586.*
32. *Killham, K. (2002): Bioindicators and Sensors of Soil Health and the Application of Geostatistics. In: Burns, R.G., Dick, R.P. (eds.) Enzymes in the Environment: Activity, Ecology and Applications. Marcel Dekker, Inc., USA*
33. *Kiss S, Pasca D, Dragan-Bularda M (1998) Development in Soil Science 26: Enzymology of Disturbed Soils, Elsevier, Amsterdam.*
34. *Labud, V., Garcia, C. and Hernander, T. (2007).Effect of hydrocarbon pollution on the microbial properties of sandy and a clay soil.Chemosphere, 66:1863–1871.*
35. *Li, D., Zhu, H., Liu, K., Liu, X., Leggewie, G., Udvardi, M. and Wang, D. (2002).Purple acid phosphatases of Arabidopsis thaliana.Journal of Biology and Chemistry, 277: 27772-27781.*
36. *Li, H., Zhang, Y., Zhang, C.G. and Chen, G. X. (2005).Effect of petroleum-containing wastewater irrigation on*

bacterial diversities and enzymatic activities in a paddy soil irrigation area. Journal of Environmental Quality, 34:1073-1080.

37. *Li, T., Meng, L., Herman, U., Lu, Z. and Crittenden, J. (2015). A Survey of Soil Enzyme Activities along Major Roads in Beijing: The Implications for Traffic Corridor Green Space Management. International Journal of Environmental Research and Public Health, Pp12475-12488.*

38. *Lopes, A.R., Faria, C., Prieto-Fernández, A., Trasar-Cepeda, C., Manaia, C.M. and Nunes, O.C. (2011). Comparative study of the microbial diversity of bulk paddy soil of two rice fields subjected to organic and conventional farming. Soil Biology and Biochemistry, 43 (1):115-125.*

39. *Margesin, R and Shinner, F. (1997). Efficiency of indigenous and inoculated cold adapted soil microorganism for biodegradation of diesel for Alpine soil, Applied and Environmental Microbiology, 63: 2660-2664.*

40. *Margesin, R. G., Walter, A. and Scheinner, F. (2000).The impact of hydrocarbon remediation on enzyme activity and microbial properties of soil.ActaBiotechnologica, 20: 313-333.*

41. *Margesin, R., Zimmerbauer, A. and Schinner, F. (2000).Monitoring of bioremediation by soil biological activities.Chemosphere 40: 339–346*

42. *Martinez-Salgado, M. M., Gutierrez-Romero, V., Jannsens, M. and Ortega-Blu, R. (2010). Biological soil quality indicators: A Review. In: Mendez-Vilas, A. (ed.) Current Research,Technology and Education Topics in Applied Microbiology and Microbial Biotechnology. Formatex.1(2): 319-328.*

43. *Megharaj, M., Singleton, I., McClure, N. and Naidu, R. (2000). Influence of petroleum hydrocarbon contamination on microalgae and microbial activities in a long term contaminated soil. Archives of Environmental Contamination and Toxicology, 38: 439–445.*

44. *Mclean, E.O. (1965). Aluminium. In: Methods of Soil Analysis (Ed C.A. Black). Agronomy No. 9 part 2.American Society of Agronomy.978-998.*

45. *Mikkonen, A., Kondo, E., Lappi, K., Wallenius, K. and Lindström K. (2011).Contaminant and plant-derived changes in soil chemical and microbiological indicators during fuel oil rhizoremediation with Galegaorientalis.Geoderma, 160: 336–346.*

46. *Minai-Tehrani, D. and Herfatmanesh, A. (2007). Biodegradation of aliphatic and aromatic*

47. *fractions of heavy crude oil-contaminated soil: A pilot study. Bioremediation. Journal, 11(2): 71-76.*

48. *McGill, W. B. and Nyborg, M. (1975). Reclamation of wet forest soils subjected to oil spills. Alberta Institute of Pedology, Canada, Publication. No. G-75-1.*

49. *Mukherjee, A. K., Bhagowati, P., Biswa, B. B., Chanda, A., and Kalita, B. (2017). A comparative intracellular proteomic profiling of Pseudomonas aeruginosastrain ASP-53 grown on pyrene or glucose as sole source of carbon and identification of some key enzymes of pyrene biodegradation pathway. J. Proteomics 167, 25–35. doi: 10.1016/j.jprot.2017.07.020*

50. *Nelson, D. L. and Cox, M. M. (2000).Principle of Biochemistry, Macmillan Press, London, Uk.*
51. *Nwaugo, V. O., Onyeagba, R. A., Akabugwo, E. I. and Ugbogu, O.C. (2008). Soil bacteria flora and enzymatic activities in zinc and lead contaminated soil. Journal of Biokemistri,20 (2): 77-84.*
52. *Obire, O. and Anyanwu, E. C. (2009).Impact of various concentrations of crude oil on fungal populations of soil.International Journal of Environmental Science and Technology, 6(20): 211-218.*
53. *Ohlinger, R., (1996). Dehydrogenase activity with the substrate Triphenyl-tetrazolium Chloride. In: Schinner, F., Ohlinger, R., Kandeler, E., Margesin, R., (eds.) Methods in Soil Biology, pg. 210-213. Springer, Berlin.*
54. *Okpokwasili, G. C. and Okolrie, B. B. (1988).Biodeterioration potentials of microorganism isolated from car engine lubricating oil. Tribology International 21: 215-220.*
55. *Okolo, J.C., Amadi, E. N. and Odu, C. T. (2005). Effects of soil treatment containing poultry manure on crude oil degradation in a sandy loam soil. Applied Ecology and Environmental Research, 3(1): 47 53.*
56. *Oliveira, A. and Pampulha, M. E. (2006).Effect of long-term heavy metal contamination on soil microbial characteristics.Journal of Bioscience and Bioengineering, 102:157-161.*
57. *Onifade, A. K., Abubakar, F. A. and Ekundayo, F. O. (2007). Bioremediation of crude oil-polluted soil in the Niger Delta area of Nigeria using enhanced natural attenuation. Research Journal of Applied Sciences. 2: 498-504.*
58. *Pena W, Trasar-Cepeda C, Gil-Sotres F, Leiros M.C (2007). Modification of the degradative capacity of a soil artificially contaminated with diesel. Chemosphere, 67: 1057–1063.*
59. *Santos, E.D.C., Silva, I.S., Simoes, T.H.N., Simioni, K.C.M and Oliveira V.M, (2012).Correlation of soil microbial community responses to contamination with crude oil with and without chromium and copper.International Journal of Biodeterioration and Biodegradation, 70: 104–110.*
60. *Saratale, G., Kalme, S., Bhosale, S. and Govindwar, S. (2007).Biodegradation of kerosene by AspergillusochraceusNCIM- 1146.Journal of Basic Microbiology, 47: 400–405.*
61. *Saterbaca, A., Toy, R.J. and Dor, P.B. (2000). Entomological and analytical assessment of effect of bioremediation on hydrocarbon contaminated soils. Environmental-Toxicology and Chemistry, 19: 2643-2652.*
62. *Saviozzi, A., Levi-Minzi R., Cardelli, R. and Riffaldi, R. (2001). A comparison of soil quality in adjacent cultivated, forest and native grassland soils. Plant and Soil, 233:251-259.*
63. *Scheinner, F., Margesin, R.G. and Ohlinger, R. (1996).Methods in Soil Biology.Springer Press. Berlin, Germany.*
64. *Seghers, D., Bulcke, R., Reheul, D., Siciliano, S. D., Top, E. M. and Verstraete, W. (2003).Pollution-induced community tolerance (PICT) and analysis of 16S rRNA genes to evaluate the long-term effects of herbicides on methanotrophic communities in soil.European Journal of Soil Science, 54: 679-684.*
65. *Serrano A., Gallego M., Gonzalez J.L, Tejada M (2008). Natural attenuation of diesel aliphatic hydrocarbons in*

contaminated agricultural soil. Journal of Environmental Pollution 151: 494–502.

66. *Serrano, A., Tejeda M., Gallego J. L. and Gonzalez, (2009). Evaluation of soil biological activity after a diesel fuel spill, Science of theTotal Environment, 404: 4056-4061.*
67. *Shaw, L.J. and Burns, R. (2006).Enzyme activity profiles and soil quality.Bloem.J., Hopkins D.W., and Benedetti. A.,(Eds.) Microbiological Methods for Assessing Soil Quality, CABI Publishing, Cambridge, MA.*
68. *Song, M., Yang, Y., Jiang, L., Hong, Q., Zhang, D., Shen, Z., et al. (2017).Characterization of the phenanthrene degradation-related genes and degrading ability of a newly isolated copper-tolerant bacterium.Environ. Pollut.220, 1059–1067. doi: 10.1016/j.envpol.2016.11.037*
69. *Srerdrup, L. G., Krogh P. H. and Nielsen, J. (2003).Toxicity of eight polycyclic aromatic compounds to red clover (Trifoliumpretense) and ryegrass.Chemosphere539(8):993-1003*
70. *Tabatabai, M. A. (1997). Soil Enzyme. In: Page, A. I., Millers, R. H. and Kenny D. R. (edition). Methods of soil analysis Part II: Chemical and Microbiology Properties.Soil Science Society of America.,. Madison, WI.*
71. *Tejada, M. (2009).Evolution of soil biological properties after addition of glyphosate, diflufenican and glyphosate diflufenicanherbicides.Chemosphere 76: 365–373.*
72. *Tejada, M., Garcia-Martinez, A., Gomez, I. and Parrado, J. (2010). Application of MCPA herbicide on soils amended with biostimulants: Short-time effects on soil biological properties. Chemosphere, 80: 1088-1094.*
73. *Truu, M., Truu, J. and Ivask, M. (2008). Soil microbiological and biochemical properties for assessing the effect of agricultural management practices in Estonian cultivated soils. European Journal of Soil Biology, 44: 231–237.*
74. *Turgay,O.C., Erdogan,E. E. and Karaca. A. (2010). Effect of humic deposit (leonardite) on degradation of semi-volatile and heavy hydrocarbon and soil quality in crude-oil contaminated soil. Environmental Monitoring and Assessment, 170: 45–58.*
75. *Udeani, C. K. T., Obroh, A. A., Okwuosa, N. C., Achukwu, U. P. and Azubike, N. (2009). Isolation of bacteria from mechanic workshops soil environment contaminated with used engine oil. African Journal of Biotechnology. 8(22): 6301-6303.*
76. *Ueno, A., Ito, Y., Yumoto, I. and Okuyama, H. (2007). Isolation and characterization of bacteria from soil contaminated with diesel oil and the possible use of these in autochthonous bioaugmentation. World Journal of Microbiology and Biotechnology. 23: 1739-1745.*
77. *Walkley, A.and Black, I. A. (1934). An examination of the Degtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents, Soil Science, 63.251-263.*
78. *Wanapaisan, P., Laothamteep, N., Vejarano, F., Chakraborty, J., Shintani, M., Muangchinda, C., et al. (2018).Synergistic degradation of pyrene by five culturable bacteria in a mangrove sediment-derived bacterial consortium.J. Hazard. Mater.342, 561–570. doi: 10.1016/j.jhazmat.2017.08.062*

79. *Wang, Y., Liang, J., Wang, J., and Gao, S. (2018). Combining stable carbon isotope analysis and petroleum-fingerprinting to evaluate petroleum contamination in the Yanchang oilfield located on loess plateau in China. Environ. Sci. Pollut. Res. 25, 2830–2841.doi: 10.1007/s11356-017-0500-6*

80. *Wang, C., Liu, X., Guo, J., Lv, Y., and Li, Y. (2018). Biodegradation of marine oil spill residues using aboriginal bacterial consortium based on Penglai 19-3 oil spill accident. China. Ecotoxicology and Environmental Safety.159, 20–27. doi: 10.1016/j.ecoenv. 2018.04.059*

81. *Wang, Y., Liang, J., Wang, J., and Gao, S. (2018). Combining stable carbon isotope analysis and petroleum-fingerprinting to evaluate petroleum contamination in the Yanchang oilfield located on loess plateau in China. Environmental Science and Pollution Research.25, 2830–2841. doi: 10.1007/s11356-017-0500-6*

82. *Xie, W., Zhou, J., Wang, H., Chen, X., Lu, Z., Yu J. and Chen, X. (2009). Short-term effects of Copper, Cadmium and Cypermethrin on dehydrogenase activity and microbial functional diversity in soils after long-term mineral or organic fertilization.Agriculture, Ecosystems and Environment, 129: 450-456*

83. *Mukut Kalita & Arundhuti Devi,Study on the Effects of Soil PH and Addition of N-P-K Fertilizer on Degradation of Petroleum Hydrocarbon Present in Oil Contaminated Soil International Journal of Chemical & Petrochemical Technology(IJCPT), Volume 2, Issue 3, November-December 2012, Pp 9-22.*

84. *Zabaloy, M. C., Garland, J. L. and Gomez, M. A. (2008).An integrated approach to evaluate the impacts of the herbicides glyphosate, 2, 4-D and metsulfuron-methyl on soil microbial communities in the Pampas region, Argentina.Applied Soil Ecology, 40:1–12.*

85. *Zahir, A.Z., Malik, M. A. R. and Arshad, M. (2001).Soil enzymes research.A Review Journal of Biological Science, 1: 299-301.*

86. *Zimenka T.G., Kartyzhova L.Y. (1986). Effect of petroleum pollution on the biological activity of a soddy-podzolic light loam soil.VestsiAkademii.*

87. *Zornoza, R., Acosta, J. A., Bastida, F., Dominguez, S.G Toledo, D.M and Faz, A (2015). Identification of sensitive indicators to assess the interrelationship between soil quality, management practices and human health.Soil Discuss., 1, 173–185.*

88. *Zheng, J., Feng, J. Q., Zhou, L., Mbadinga, S. M., Gu, J. D., and Mu, B. Z. (2018). Characterization of bacterial composition and diversity in a long-term petroleum contaminated soil and isolation of high-efficiency alkane-degrading strains using an improved medium. World J. Microbiol.Biotechnol.34:34. doi: 10.1007/s11274-018-2417-8*