



Bacteriological and Physicochemical Properties of Petroleum-Contaminated Soil Collected from a Mechanic Site in Abuja, North-Central Nigeria

Kawo Abdullahi Hassan¹, Yahaya Sani¹, Olawore Yemisi Ajoke^{1,2,*}, Ogidi Jonathan Ajisafe²

¹Department of Microbiology, Faculty of Life Sciences, Bayero University, Kano, Nigeria

²Department of Applied Mathematics, National Mathematical Centre, Sheda-Abuja, Nigeria

Email address:

olaworeyemisi1@yahoo.com (O. Y. Ajoke)

*Corresponding author

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Abstract: Elevated levels of petroleum hydrocarbon and certain heavy metal compounds in soil samples due to environmental and manmade operations can amount to concentrations considered as toxic. Soil samples from 3 different locations, 30 meters from each other were collected and assessed for polycyclic aromatic hydrocarbon (PAH), total petroleum hydrocarbon (TPH), selected heavy metal concentrations, pH, Electrical Conductivity, texture, organic matter, moisture content and bacterial population. The results on the test sites showed that pH range for the workshop soil samples was between 5.08-5.45 in comparison to the control 6.74. Data obtained also revealed that the tested site A alone contained lead (44.91 mg/g) and cadmium (0.01 mg/g). The heavy metal content for sites A, B and C were higher than that of the control site D for which the enrichment factors were determined for sodium (1.07, 3.34, 4.12), copper (2.73, 2.63, 1.57), iron (9.84, 10.67, 9.21), zinc (4.78, 2.58, 2.98), nickel (1.44, 1.11, 1.56) and manganese (2.57, 2.23, 3.77) respectively. The moisture content and electrical conductivity ranged between 44.7-46.5% and 985.6-1124.7 ($\mu\text{S}/\text{cm}$) respectively as opposed to 44.9%, 846.3 ($\mu\text{S}/\text{cm}$) for the control. Total bacterial enumeration revealed a count of 2.28×10^4 (cfu/g), 2.01×10^4 (cfu/g), 1.98×10^3 (cfu/g) and 2.71×10^5 (cfu/g) at sites A, B, C and D respectively. The presence of hydrocarbon and heavy metal pollutants due to the activities at the mechanic workshop resulted in change of known physicochemical properties which in turn affects the ecology as well as life forms in the area.

Keywords: Petroleum Hydrocarbons, Polycyclic Aromatic Hydrocarbons, Heavy Metals, Physicochemical Properties, Mechanic Site, Abuja

1. Introduction

Industrialization and global expansion has in recent times witnessed the increase sales of petroleum products that serve the use of automotive devices around the world. Although these products serve the needs of mankind, their disposal presents a unique challenge to the environment for which growing concerns of their effect on human health, damages terrestrial and aquatic life forms, destruction of different ecosystems, depletion of soil fertility, etc has sparked worldwide protests [1-7]. Avenues by which petroleum products slip or enter the environment is not limited to

pipeline leakage or vandalism and tanker accidents spills, inappropriate handling of spent lubrication oils (engine oil) particularly in developing countries represents the most common avenue in developing countries like Nigeria [8-9]. Used automotive engine oils are known to contain several heavy metals as well as polycyclic aromatic hydrocarbons (PAHs) both of which leach into groundwater from the soil thereby entering the food chain, thus initiating the development of carcinogens and mutagens in mankind [10].

Like most petroleum products, used engine oils comprises not only of linear and polyaromatic structures, it also would contain several heavy metals which it absorbed over time via the combustion activities of engine parts, thereby varying

degrees of complexity for such oils that are inadequately disposed of in mechanical workshops [11]. The distribution and accumulation of heavy metals like Cadmium, Copper, Chromium, Iron, Lead, Manganese, Nickel, etc within any environment is important as at elevated levels, these metals wield peculiar hazards to mankind whereby they leach into other locations where plants are grown for consumption. Reports suggest that upon ingestion of plants tainted with elevated levels of heavy metals, these contaminants gain entry to the blood stream and are circulated to other parts of the body where they disrupt metabolic functions, weaken the immune system, impede cell signaling, thereby triggering a cascade of molecular events that preempts the production of reactive oxygen species, development of carcinomas, and onset of reproductive defects [12-13].

Bombardment of the soil with frequent quantities of used engine oil distorts the quality and functional characteristics of the soil whereby its ability to support life diminishes such that its texture, compaction conductivity, ability to retain water, etc become impaired [14-15]. Studies show that plant survival and microbial populations generally decreases as the concentration of petroleum products in the soil increases over time [16-17]. Soil microorganisms are particularly important as these serve in bolstering soil nutrient cycles, soil fertility and the flow of organic matter, decomposition and transformation [18-19]. As a biological indicator of changes to the environment, soil microorganisms can be used to quantify the physicochemical properties of the soil as they are highly susceptible to changes including the presence of pollutants.

In the Abuja Metropolis of Nigeria, the Apo mechanic village is one of the largest automotive workshops in the city which utilizes a mixture of engine oil, petrol, diesel and other petroleum products daily. They uniquely harden nature of the soil within this environment owing to the large volumes of hydrocarbons disposed of would undoubtedly have affected the physiological properties of the soil as characterized by its hard and darkened soil texture.

As the population of the city increases, it is expected that the number of vehicles, hence used engine oil amongst other petroleum products would be discharged into the environment. These necessitates the need to monitor the exposure of such hydrocarbons and associated heavy metals to the soil as such studies would aid the local environmental authorities to establish safety parameters and instill effective waste management practices pertaining of spent automotive fluids within automotive workshops in order to safeguard the environment.

This study aimed at evaluating the physicochemical properties of soil contaminated with spent oils from a mechanic workshop in Abuja.

2. Materials and Methods

Soil samples were collected randomly in sterile polystyrene sampling containers using a sterile spatula from the center of motor oil-stained patches from three (3) points

in the workshops by scooping to about 20cm from the Apo mechanic village workshop in Abuja, Nigeria. The sampling points were 30 meters away from each other in either direction. Samples were also collected from a control site 100m away from the mechanic workshop. The containers were labelled properly and immediately transported to the Sheda Science and Technology Complex (SHESTCO) laboratories for analyses. Sampling was done during the rainy season following a week-long period of no rainfall.

2.1. GC-MS Determination of Hydrocarbons

10g of homogenized sediment sample was weighed into 250ml Teflon bottle. 20ml of 1:1 acetone: dichloromethane was added and the mixture was sonicated in an ultrasonic bath at 70°C for 30 min. Organic layer was decanted into a clean beaker/round-bottom flask, further dried with sodium sulfate and clean-up procedure using silica gel column carried out. The sample extract was then concentrated to 2ml using a rotary evaporator prior to analysis using a Gas Chromatography mass spectrometer (GC-MS).

PAHs standard, 1000ppm (Catalog Number: H-QME-01) containing 23 environmental PAHs components was purchased from AccuStandard was employed. Five (5) point serial dilution calibration standards (1.00, 5.00, 10.00, 50.00, 100.00ppm) was prepared from the stock and used to calibrate the GC-MS.

Determination of the levels of PAHs in the sample was carried out using GC-MS (Agilent 7820A- 5975C inert mass spectrometer, Agilent Technologies®) by operating MSD in selective ion monitoring (SIM) and Scan mode to ensure low level detection of the target constituents. The stationary phase of separation of the compounds was HP-5 capillary column coated with 5% Phenyl Methyl Siloxane (30m length x 0.32mm diameter x 0.25µm film thickness) (Agilent Technologies). The carrier gas was Helium used at constant flow of 1.48 mL/min at an initial nominal pressure of 1.49 psi and average velocity of 44.22 cm/sec. 1µL of the samples were injected in splitless mode at an injection temperature of 300°C. Purge flow to spilt vent was 15.0 mL/min at 0.75 min with a total flow of 16.67 mL/min; gas saver mode was switched off. Oven was initially programmed at 40°C (1 min) then ramped at 12°C/min to 300°C (10 min). Run time was 32.67 min with a 3 min solvent delay. The mass spectrometer was operated in electron-impact ionization mode at 70eV with ion source temperature of 230°C, quadrupole temperature of 150°C and transfer line temperature of 300°C [20-21].

2.2. AAS Determination of Heavy Metals

The metal analysis was determined using an Atomic Absorption spectrometer (iCE 3000, Thermo fisher). 0.5g of each dried soil was digested with concentrated HNO₃, H₂SO₄ and H₂O₂ (2:6:6) as prescribed [13]. The blanks were run in a set and samples were analyzed at the Chemistry Advanced research Centre, SHESTCO, Abuja, Nigeria. Elemental analysis of the solution obtained was then performed using

an atomic absorption spectrophotometer (AAS) at an appropriate wavelength, temperature and lamp-current for the different elements. The following elements were determined; Cadmium (Cd), Calcium (Ca), Copper (Co), Iron (Fe), Lead (Pb), Magnesium (Mg), Manganese (Mn), Nickel (Ni), Potassium (K), Sodium (Na), and Zinc (Zn). The concentrations of heavy metals were expressed as mg/g.

2.3. Estimation of Total Bacterial Population

The population of bacteria within the contaminated and controlled soil samples were determined using known techniques [22]. 1g of each soil sample was suspended into 10 ml of sterile distilled water and was aseptically serially diluted further up to 10^{-7} dilution. 0.1ml aliquots from each diluted soil suspension was poured onto nutrient agar plates using the spread plate technique. Plates were incubated for 24-72 hours at 37°C and the results recorded. All tests were done in triplicate.

2.4. Soil Physicochemical Analysis

Soil samples were air dried for 72 hours at room temperature. The dried samples were ground using a mortar and pestle then sieved with a 2.0mm mesh. 1kg of each soil sample was weighed into the crucible and heated in an oven at 105°C overnight. Sample measurements were taken after the crucibles were cooled in a desiccator for 1-2 hours after which the percentage moisture content (% MC) was calculated by calculating the loss in drying as a fraction of the initial mass of sample and multiplied by 100 [23]. The electrical conductance of the soil sample was determined by preparing a 1:2 slurry of 10g fresh soil in 20ml deionised water. Measurements were taken using conductivity meter (Systronics-304) at 25°C.

Soil pH was determined for all four samples using a separate 1:2 slurry of 10g soil sample in 20ml deionised water. The slurry was allowed to stand for 10 minutes then the pH readings was recorded using a digital pH meter (Jenway model 3015). Each suspension was stirred vigorously using a glass rod immediately prior to pH determination [13]. The Total Organic Matter content for all studied samples was determined using known methods [24]. 10 ml of potassium heptaoxidichromate (IV) solution ($K_2Cr_2O_7$) was pipetted into a 250ml conical flask containing 1g of sieved, air-dried, ground soil sample. The solution was mixed via gentle swirling followed by the addition of 20ml concentrated tetroxosulphate (VI) acid. The flask was swirled vigorously for 10 minutes and the mixture was allowed to stand for 30 minutes. 100 ml of distilled water was added into the flask followed by the addition of 4 drops of Ferroin indicator. Then the mixture was titrated with 0.5 M iron (II) sulphate solution, added by single drops until the colour of the mixture changed to light green>dark green>brownish red-end. A blank titration was performed similarly without soil to standardize the $K_2Cr_2O_7$. The soil organic content in each treatment soil was determined. The values obtained for the organic content was then used to determine

the soil organic matter content of the studied samples, using the 0.58 constant.

3. Results

3.1. Hydrocarbon Content Analysis

Three soil samples obtained from different locations within the studied sites (Apo mechanic workshop) were given the designation A, B, and C respectively while that of the control was denoted as D. chromatographic analysis of these soils revealed the presence of 19-20 different polycyclic aromatic hydrocarbons at different concentrations (Table 1). The presence of the 3-ringed Acenaphthylene, Acenaphthene and Fluorene compounds, all of which were absent in D were identified at varied concentrations in A, B and C.

3.2. Heavy Metal Content Analysis

The assessment of the heavy metals present and their concentrations in A, B, C and D revealed a higher concentration of all tested metals in A and a generally higher content in B and C in comparison to the control (Table 2). Lead and Cadmium were not detected in sites B, C and D. the highest recorded concentrations, 7.68 – 81.92 (mg/g) was for Iron. Enrichment of the soil by each heavy metal was calculated (Table 3).

Table 1. Hydrocarbon content and concentration of soil samples.

Components	Site A (mg/kg)	Site B (mg/kg)	Site C (mg/kg)	Control (mg/kg)
Naphthalene	0.86	0.36	0.00	0.29
Acenaphthylene	0.40	0.36	0.93	0.00
Acenaphthene	0.74	0.34	0.37	0.00
Fluorene	1.56	0.85	0.89	0.00
Phenanthrene	6.08	5.16	1.18	0.36
Anthracene	11.94	2.15	1.75	0.74
Fluoranthene	1.99	6.41	0.46	0.58
Pyrene	3.73	1.97	2.68	0.96
Benzo [c] phenanthrene	4.47	5.94	2.41	1.04
Benz [a] anthracene	4.01	4.50	4.13	0.85
Chrysene	9.88	13.09	10.73	1.47
Benzo [e] pyrene	4.64	12.95	15.28	3.25
Benzo [k] fluoranthene	36.29	27.53	118.56	1.69
3-Methylcholanthrene	44.35	43.46	82.59	9.87
Indeno [1, 2, 3-cd] pyrene	11.38	29.43	80.22	3.76
Dibenz [a, h] anthracene	18.97	11.13	16.03	10.41
Benzo [ghi] perylene	13.51	155.10	97.48	12.12
Dibenzo [a, h] pyrene	1.94	15.82	6.57	6.17
Dibenzo (a, i) pyrene	8.09	6.97	17.00	2.19
Dibenzo (a, l) pyrene	7.25	6.30	14.83	4.49
Benzo [a] pyrene	39.91	52.04	85.80	0.76
Total PAH (mg/kg)	231.999	401.860	559.905	61.003
TPH (mg/kg)	9,729	115,933	123,874	652

Table 2. Heavy Metal content and concentration of soil samples.

Elements	Site A (mg/g)	Site B (mg/g)	Site C (mg/g)	Control (mg/g)
Sodium	1.45	4.51	5.56	1.35
Potassium	0.36	0.59	0.77	0.21
Zinc	3.06	1.65	1.91	0.64

Elements	Site A (mg/g)	Site B (mg/g)	Site C (mg/g)	Control (mg/g)
Lead	44.91	-	-	-
Copper	0.82	0.79	0.47	0.30
Nickel	0.13	0.10	0.14	0.09
Cadmium	0.01	-	-	-
Iron	75.59	81.92	70.72	7.68
Magnesium	5.76	3.66	22.45	1.82
Calcium	13.35	2.92	1.68	1.07
Manganese	3.93	3.41	5.77	1.53

Table 3. Enrichment Factor (EF) of Heavy Metals in Polluted soil.

Elements	Site A (EF)	Site B (EF)	Site C (EF)
Sodium	1.07	3.34	4.12
Potassium	1.71	2.81	3.67
Zinc	4.78	2.58	2.98
Lead	44.91	-	-
Copper	2.73	2.63	1.57
Nickel	1.44	1.11	1.56

Table 4. Physicochemical and Biological Properties of soil samples.

Parameter	Site A	Site B	Site C	Site D
Moisture Content (%)	45.2	44.7	46.5	44.9
Organic Matter (%)	2.1	7.41	13.5	1.2
pH	5.45	5.22	5.08	6.74
Conductivity ($\mu\text{s cm}^{-1}$)	985.6	1024.3	1124.7	846.3
Texture Class	Sandy Loam	Sandy Loam	Sandy Loam	Sandy Clay
Bacterial count (CFU/g)	2.28×10^4	2.01×10^4	1.98×10^3	2.71×10^5

4. Discussion

The inadvertent release of petrochemical agents directly to the soil triggers a chain of events which negatively impacts on biotic and abiotic elements within the environment [25]. Crude oil and its derivatives are composed of aliphatic, oleic, naphthenic and aromatic hydrocarbons, which modify physical and chemical properties of soil and its structure [26]. These compounds are largely responsible for changes in soil fertility. Soil polluted by petroleum-based products losses its biological activity and may take a considerable time to recover without remedial measures [27].

In this study, the total petroleum hydrocarbons (TPH) and total concentration of polycyclic aromatic hydrocarbons (PAH) was found to be 559.9 (mg/kg), 401.9 (mg/kg), 232 (mg/kg) and 61 (mg/kg) for sites C, B, A and D, the latter representing the uncontaminated control site (Table 1). The elevated values of both TPH and PAH recorded in sites A, B and C in comparison to the control site is as a direct result of soil contamination with automobile fluids via the activities of the mechanic workshop on the site. The deleterious effect of contamination is worsened by the presence toxic components of TPH like phenols, chlorinated hydrocarbons, etc all of which retard the growth of indigenous microorganisms [28]. The prevalence of Naphthalene (0.86, 0.36 mg/kg), Acenaphthylene (0.4, 0.36, 0.93 mg/kg), Fluorene (1.56, 0.85, 0.89 mg/kg), Phenanthrene (6.08, 5.16, 1.18 mg/kg), Anthracene (11.94, 2.15, 1.75 mg/kg) and Pyrene (3.73, 1.97, 2.68 mg/kg) in soil from sites A, B and C respective is suggestive of incomplete combustion activities coupled with

Elements	Site A (EF)	Site B (EF)	Site C (EF)
Cadmium	0.01	-	-
Iron	9.84	10.67	9.21
Magnesium	3.16	2.01	12.34
Calcium	12.48	2.73	1.57
Manganese	2.57	2.23	3.77

3.3. Physicochemical Characterization

pH, moisture content, organic matter, electrical conductivity and texture measurements for all studied samples was examined (Table 4). Samples from sites A, B and C were more acidic in nature than that of D which leaned more towards pH neutral. The organic matter and moisture content was highest in C. Conductivity measurements were high for all studied soil samples.

Estimations of the bacteria populations in each studied site was enumerated (Table 4).

the release of worn out automotive materials and coatings which is should coincide with an above normal heavy metal presence. The resultant TPH values aligns with that obtained for PAH thus suggesting a higher but inappropriate rate of petroleum product disposal emanating from site C or the highest accumulation polluting agents at site C quickly followed by site B (Table 1).

Zinc, Lead, Iron, Copper, Cadmium and Cobalt are heavy metals common found in automobile workshop environments [29-30]. The presence of these heavy metals at high concentrations in busy mechanic workshop environments is not uncommon as depicted in the data obtained in this study (Table 2). In this study, all nutritional and environmental metals tested in the contaminated sites were higher than that of the control. Any vegetative wildlife within such an environment easily bio-accumulates these heavy metals which is eventually consumed by man via the natural food chain. While heavy metals like iron performs vital metabolic functions to all life forms, it poses a health hazard for humans, animals, plants and microorganisms at elevated concentrations including but not limited to constipation, bloody diarrhea, hemosiderosis, hemochromatosis, cirrhosis, increased incidence of hepatoma, diabetes, cardiac failure, etc. consumption of plants or animals with elevated levels of calcium leads to anorexia, lethargy, nausea, vomiting, constipation, arrhythmias, hypertension, myopathy, calcification of heart, lungs, kidneys, etc. while elevated levels of zinc causes copper deficiency, reduced HDL and immune function, stomach cramps, nausea and vomiting, pancreas damage, gastrointestinal irritation and microcytic anaemia, elevated levels of copper in food sources causes

liver damage, weakness, diarrhea and gastric pain in lieu of elevated manganese which causes Psychosis and Parkinsonian syndrome [31]. Data obtained further showed that the most significant enrichment factor values for the detected heavy metals was for lead and iron across all three contaminated sites (Table 3). An enrichment factor values in excess of 1 specifies an increased availability and distribution of metals in contaminated soil, thus aggregating the metal accumulation in plants species grown in contaminated soil [32].

Data obtained (Table 4) revealed that the pH of the control site was 6.74 which was nearly neutral while that of the studied sites were more acidic with an increase in pH units of 0.14 between C-B and 0.23 pH units between B to A. This result is aligned with the concentrations of PAH and TPH whereby petroleum products in the soil triggered an increase in the pH due to the sequestering of organic acids coupled with the fracturing of the gaseous exchange balance for which carbon dioxide is retained more in the soil [30]. That study also highlighted that the binding of petrochemical oils to the soil matrix led to the leaching of fundamental salts and basic ions which in turn affects the pH. Distortions to the soil's pH balance would in effect alter the nutrient accessibility for vegetative and microbial life within, resulting in a decrease in both plant and microbial population as well as diversity [30]. Further data obtained revealed that the higher the concentration of TPH and PAH in the different sites, the higher the electrical conductivity (1124.7, 1024.3, 985.6, 846.3 $\mu\text{s cm}^{-1}$) and organic matter (13.5, 7.41, 2.1, 1.2%) for site C, B, A and D respectively (Table 4).

Analysis of the total microbial population suggested that due to the high concentrations of petroleum oils in the soil samples, the microbial population reduced in environments with elevated hydrocarbon presence (Table 4). The lower bacterial count between sites A (2.28×10^4 CFU/ml), B (2.01×10^4 CFU/ml), C (1.98×10^3 CFU/ml) and D (2.71×10^5 CFU/ml) depicts the effects of petroleum hydrocarbons on the microbial flora in the soil (Table 4). It has been reported that the elevated concentrations of PAH and TPH in soils would cause the excess retention of carbon dioxide in particular, thus affecting the porosity of the soil environment as well as triggering the increase in soil pH, all of which would encumber microbial metabolism, hence affecting the growth and population size [33].

5. Conclusion

Data obtained in this study has shown that the activities from the mechanic workshop caused an increase in the TPH from site A (9,729 mg/kg), site B (115,933 mg/kg) and site C (123,874 mg/kg) in comparison to the control site D (652 mg/kg). The effects of uncontrolled disposal of petroleum chemicals also resulted in higher concentrations of polycyclic aromatic hydrocarbons (PAH) at site A (231.999 mg/kg), site B (401.86 mg/kg) and site C (559.905 mg/kg) as compared to the control site D (61.003 mg/kg). The presence of these

hydrocarbons within the environs around the mechanic workshop is deleterious to the wildlife, thus making the region not only ecologically unsustainable but hazardous for human habitation in the long run. The overall elevated concentrations of the assayed environmental heavy metals further projects the damaging effects of the petroleum waste practices within the vicinity of the mechanic workshop which together with the concentrations of the TPH and PAH (Tables 1-2) would alter the physicochemical characteristics of the samples which would eventually leaches into the aquifers from which it may be directly consumed by mankind. The enrichment factors for each studied heavy metal verifies their introduction to the soil from the automobiles (Table 3). Notable physicochemical features included a more acidic environment, increased organic matter, higher electrical conductivity and lower microbial load by way of colony forming units (Table 4). Automotive waste oils altered the physical, chemical and microbial characteristics of the soil which would affect plant roots and subsequently impede nutrient availability and uptake from the soil. This finally may lead to decrease in crop yield.

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References

- [1] Bamidele J., and Igiri A., (2011). Growth of seashore paspalum (*Paspalum vaginatum* L.) in soil contaminated with crude petroleum oil. *Journal of Applied Sciences and Environmental Management*, 15: 303-306.
- [2] Oluyeye J. O., Odere J. O., and Kayode J., (2011). Effect of organic manure on the aliphatic and aromatic compositions of diesel-oil contaminated soil. *Annals of Science, Engineering and Technology*, 1: 22-30.
- [3] Oyediji A. A., Adebisi A., Omotoyinbo M., and Ogunkunle C., (2012). Effect of crude oil-contaminated soil on germination and growth performance of *Abelmoschus esculentus* L. Moench- a widely cultivated vegetable crop in Nigeria. *American Journal of Plant Sciences*, 3: 1451-1454.
- [4] Sharonova N., and Breus I., (2012). Tolerance of cultivated and wild plants of different taxonomy to soil contamination by kerosene. *Science of the Total Environment*, 424: 121-129.
- [5] Ewetola E. A., (2013). Effect of crude oil pollution on some soil physical properties. *Journal of Agriculture and Veterinary Science*, 6: 14-17.
- [6] Hentati O., Lachhab R., Ayadi M., and Ksibi M., (2013). Toxicity assessment for petroleum-contaminated soil using terrestrial invertebrates and plant bioassays. *Environmental Monitoring and Assessment*, 185: 2989-2998.

- [7] Efe S. I., and Elenwo E. I., (2014). Phytoremediation of crude oil contaminated soil with *Axonopus compressus* in the Niger Delta region of Nigeria. *Natural Resources*, 5: 59-67.
- [8] Kayode J., Olowoyo O., and Oyediji A. A., (2009). The effects of used engine oil pollution on the growth and early seedling performance of *Vigna unguiculata* and *Zea mays* L. *Research Journal of Soil Biology*, 1: 15-19.
- [9] Ogunbayo A. O., Bello R. A., and Nwagbara U., (2012). Bioremediation of Engine Oil Contaminated Site. *Journal of Emerging Trends in Engineering and Applied Sciences*, 3: 483-489.
- [10] Boonchan S., Britz M. L., and Stanley G. A., (2000). Degradation and Mineralization of high-molecular weight polycyclic aromatic hydrocarbons by defined fungal-bacterial co-cultures. *Applied Environmental Microbiology*, 66: 1007-1019.
- [11] Moubasher H. A., Hegazy A. K., Mohamed N. H., Moustafa Y. M., Kabil H. F., Hamad A. A., (2015). Phytoremediation of soils polluted with crude petroleum oil using *Bassiascoparia* and its associated rhizosphere microorganisms. *International Journal of Biodeterioration and Biodegradation*, 98: 113-120.
- [12] Merril J. C., Morton J. J. P., and Soileau S. D., (2007). Metals: cadmium. In: Hayes AW (ed) Principles and methods of toxicology, 5th edn. Taylor and Francis, London, pp 665-667.
- [13] Useh M. U., Etuk-Udo G. A., and Dauda M. S., (2015). Evaluating the Physico-chemical Properties and Heavy Metals in Soils of Municipal Waste Dumpsites at Kubwa, Abuja, Nigeria. *Journal of Chemistry and Chemical Sciences*, 5: 654-662.
- [14] Hreniuc M., Coman M., and Cioruța B., (2015). Consideration regarding the soil pollution with oil products in Sacel-Maramures. International Conference of scientific paper AFASES. Brasov: 28-30.
- [15] Gennadiev A., Yu N., Pikovskii I., Tsibart A. S., and Smirnova M. A., (2015). Hydrocarbons in Soils: Origin, Composition, and Behavior Review). *European Journal of Soil Science*, 48:1076-1089.
- [16] Gaskin S. E., and Bentham R. H., (2010). Rhizoremediation of hydrocarbon contaminated soil using Australian native grasses. *Science of the Total Environment*, 408:3683-3688.
- [17] Masakorala K., Yao J., Chandankere R., Liu H., Liu W., Cai M., and Choi M. F., (2014). A combined approach of physicochemical and biological Methods for the characterization of petroleum hydrocarbon contaminated soil. *Environmental Science and Pollution Research*, 21: 454-463.
- [18] Schneider T., Keiblinger K. M., Schmid E., Sterflinger-Gleixner K., Ellersdorfer G., Roschitzki B., Richter A., Eberl L., Zechmeister-Boltenstern S., and Riedel K. (2012). Who is who in litter decomposition? Metaproteomics reveals major microbial players and their biogeochemical functions. *The ISME Journal*, 1: 1-14.
- [19] Massenssini A. M., Bonduki V. H. A., Melo C. A. D., Tótola M. R., Ferreira F. A., and Costa, M. D. (2015). Relative importance of soil physico-chemical characteristics and plant species identity to the determination of soil microbial community structure. *Applied Soil Ecology*, 91: 8-15.
- [20] Wang Z., and Stout S. A., (2007). Oil Spill Environmental Forensics: Fingerprinting and Source Identification. Academic Press.
- [21] Wang Z., and Stout S. A., (2016). Standard Handbook Oil Spill Environmental Forensics: Fingerprinting and Source Identification. Elsevier/Academic Press.
- [22] Lily M. K., Bahuguna A., Dangwal K., and Garg V., (2009). Degradation of benzo [a] pyrene by a novel strain *Bacillus subtilis* BMT4i (MTCC 9447). *Brazilian Journal of Microbiology*, 40: 884-892.
- [23] Pathak H., Bhatnagar K., and Jaroli D. P., (2011). Physico-chemical Properties of Petroleum Polluted Soil Collected from Transport Nagar (Jaipur). *IJFALS*, 1: 84-89.
- [24] Ibitoye A. A., (2006). *Laboratory Manual on Basic Soil Analysis*. 2nd Edition, Foladave Nigeria Ltd., Akure, Nigeria, pp. 70.
- [25] Bamidele J., and Igiri A., (2011). Growth of seashore paspalum (*Paspalum vaginatum* L.) in soil contaminated with crude petroleum oil. *Journal of Applied Sciences and Environmental Management*, 15: 303-306.
- [26] Sharma S., and Pathak H., (2017). Physico-chemical properties of petroleum contaminated soil Collected from arid region of Rajasthan (Churu). *International Journal of Pharma and Biosciences*, 8: 926-932.
- [27] Taneer F. B. G., and Kinako P. D. S., (2008). Comparative studies of bio-stimulation and phytoremediation in the mitigation of crude oil toxicity in tropical soil. *Journal of Applied Sciences and Environmental Management*, 12: 143-147.
- [28] Ujowundu C. O., Kalu F. N., Nwaoguikpe R. N., Kalu O. I., Ihejirika C. E., Nwosunjoku E. C., and Okechukwu R. I., (2011). Biochemical and physical characterization of diesel petroleum contaminated soil in southeastern Nigeria. *Research Journal of Chemical Science*, 1: 57-62.
- [29] Khan S. R., Kumar J. I. N., Kumar R. N., and Patel J. G., (2013). Physicochemical properties, heavy metal content and fungal characterization of an old gasoline-contaminated soil site in Anand, Gujarat, India. *Environmental and Experimental Biology*, 11: 137-143.
- [30] Cheraghi M., Sobhanardakani S., Lorestani B., Merrikhpour H., and Parvizi Mosaed H., (2015). Biochemical and Physical Characterization of Petroleum Hydrocarbon Contaminated Soils in Tehran. *Journal of Chemical Health Risks*, 5: 199-208.
- [31] Prashanth L., Kattapagari K. K., Chitturi R. T., Baddam V. R. R., and Prasad L. K., (2015). A review on role of essential trace elements in health and disease. *Journal of Dr. NTR University of Health Sciences*, 4: 75-85.
- [32] Gupta S., Nayek S., Saha R. N., and Satpati S., (2008). Assessment of heavy metal accumulation in macrophyte, agricultural soil and crop plants adjacent to discharge zone of sponge iron factory. *Environmental Geology*, 55: 731-739.
- [33] Khan A., Khan S., Khan M. A., Qamar Z., and Waqas M., (2015). The uptake and bioaccumulation of heavy metals by food plants, their effects on plants nutrients, and associated health risk: a review. *Environmental Science and Pollution Research International*, 22: 13772-13799.