



Gorgan University of  
Agricultural Sciences  
and Natural Resources



## Assessment of petroleum sludge pollution risk on some characteristics of active carbon-treated soil

Roghayeh Karimpoor<sup>1</sup>, Soheila Ebrahimi<sup>2\*</sup> , Elham Malekzadeh<sup>3</sup>

<sup>1</sup> M.Sc. graduate, Department of Soil Sciences, Faculty of Soil and Water Engineering, Gorgan, University of Agricultural Sciences and Natural Resources, Gorgan, Iran

<sup>2</sup> Assistant Professor, Department of Soil Sciences, Faculty of Soil and Water Engineering, Gorgan, University of Agricultural Sciences and Natural Resources, Gorgan, Iran, Email: sohebrahimi@gmail.com

<sup>3</sup> Assistant Professor, Department of Soil Sciences, Faculty of Soil and Water Engineering, Gorgan, University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Article Info	Abstract
<b>Article type:</b> Research Article	<p>The objective of this study was to investigate the effect of various active carbon weights (100 and 150 g) and sizes (0.3, 1.5 and 4 mm) on the remediation of soil hydrocarbon pollution and monitor the negative impacts of petroleum hydrocarbons on parameters such as C/N, electrical conductivity (EC) and hydrophobicity of aggregates in petroleum polluted soil. This pilot study was conducted over 60 days as a split-plot in time experiment based on a completely randomized design with three replicates and included seven treatments (W100S0.3, W100S1.5, W100S4, W150S0.3, W150S1.5, W150S4, C), in which w and s indicated active carbon weight (g) and size (mm), respectively. The results showed that the application of active carbon at smaller sizes resulted in a significant decrease in the concentrations of C, N, C/N, pH, and total petroleum hydrocarbons (TPH). Active carbon treatment with 150 g weight and 0.3 mm size was the most effective treatment in TPH biodegradation, reduction of organic carbon content and C/N ratio in the polluted soil. Also, this treatment decreased aggregate hydrophobicity and dispersible clay percentage by TPH degradation.</p>
<b>Article history:</b> Received: September 2023 Accepted: MAY 2024	
<b>Corresponding author:</b> maleki@sku.ac.ir	
<b>Keywords:</b> Bioremediation Hydrocarbon Oil pH polluted soil	

**Cite this article:** Karimpoor, Roghayeh; Ebrahimi, Soheila; Malekzadeh, Elham. 2024. Assessment of petroleum sludge pollution risk on some characteristics of active carbon-treated soil. *Environmental Resources Research*, 12(1), 167-182.



© The Author(s).

DOI: 10.22069/IJERR.2024.20987.1394

Publisher: Gorgan University of Agricultural Sciences and Natural Resources

### Introduction

The petroleum industry is the main pillar of economy in Iran and petroleum-rich countries such as Iran are always at risk of soil and water pollution by petroleum and its derivatives which include hydrocarbon and chemical solutions that negatively affect the environment. There are many concerns and issues associated with the

massive amount of chemicals and petroleum derivatives released into the environment each year (Ayoubi et al., 2020). In recent decades and due to the expansion of petroleum refineries across the world, great amounts of dense solid waste called petroleum sludge are produced during the various stages of production, transport, and refining.

Petroleum sludge is solid waste that is stored for long terms in pits close to petroleum production facilities and pollutes lower soil layers (Das et al., 2018). Petroleum sludge is regarded as hazardous waste in many countries and its disposal requires effective refining technologies (Liu et al., 2019). Over time, petroleum sludge undergoes different biological and non-biological processes such as limited microbial degradation, oxidation, and sublimation, leading to various levels of pollution (Cai et al., 2021). Petroleum pollution in the soil leads to reduced plant and microbe diversity, poor soil fertility, impairment of ecological balance, disruption, or delay in crop germination, decreased chlorophyll content, and necrosis in some crops (Sui et al., 2021). The n-alkanes, derived alkanes, and cyclo-alkanes present in petroleum sludge can cause many neurological, respiratory, and kidney disorders (Allamin et al., 2021). The main reason for this issue is the high concentration of a complex emulsion of different petroleum hydrocarbons (Tarno et al., 2011) which serve as energy and carbon source for microorganisms. Petroleum sludges have a pH range of 6.5 to 7.5 and are defined as a stable emulsion of water, solid materials, petroleum hydrocarbons and heavy metals (Hu et al., 2013).

Total petroleum hydrocarbons in petroleum sludge are considered between 5 to 86.2 weight percentage, but it is mostly between 15 to 50% percent. Nitrogen concentration in petroleum sludge is usually less than 3. Sulphur concentrations range between 3 to 10% and oxygen content is less than 4.8% (Hu et al., 2013). Many parameters such as pH, texture, moisture, nutrients- especially nitrogen and phosphorous- and organic matter directly affect the bioavailability of the pollutant and consequently, its degradation. Therefore, the removal of pollutants in different soils takes place at different rates (Vickers, 2017). Bioremediation involves the enhancement of the natural ability of microorganisms to degrade pollutants and decrease the risk of pollution (Azubuike et al., 2016). This process occurs via the biodegradation of the pollutant by adding degrading materials or biostimulation by

nutrients (Chikere et al., 2015; Beaker et al., 2016). The addition of nutrients rich in mineral or organic nitrogen to stimulate microbial activity and degrade petroleum hydrocarbons is reported as an effective method for bioremediation (Agarry et al., 2010). Nitrogen and phosphorous affect microbial propagation as nutrient sources for the growth of bacteria cells. Bacteria and fungi possess the ability to modify natural and synthetic pollutants into energy sources and non-toxic compounds during their growth or metabolic processes (Neebee, 2020).

Maintenance of tissues and soil characteristics are among the advantages of bioremediation. Moreover, bioremediation may enhance the physicochemical characteristics of soil such as aeration, pH, water retention and cation exchange capacity (Nwogu et al., 2015). Therefore, the utilization of various biological strategies is required to develop an effective bioremediation protocol to maximize the removal of petroleum pollutants (Raimondo et al., 2020). Organic matter is a key factor in aggregate stability and soil structure (Rengasamy et al., 1984). Increased organic carbon in the soil leads to a reduction in the content of dispersible clay in the soil (Dexter et al., 2008).

Petroleum breaks down soil structure and ecological function (Kumari et al., 2018) and significantly affects soil moisture, pH, total carbon, total nitrogen, exchangeable potassium, and enzyme (urease, catalase, dehydrogenase) activity (Achuba et al., 2008, Barua et al., 2011). High concentrations of petroleum in soil result in increased clay content (Osuji et al., 2006), decreased porosity, and increased impermeability and hydrophobicity (Xin et al., 2012) which inhibits the growth of plant roots and bacteria population in the soil.

Black carbon with a high specific area, properly distributed pores, and high affinity is called active carbon, which is also known as active coal (Saygili, 2019; Zhang et al., 2018). Reports show that microorganisms play a key role in the degradation of hydrocarbon compounds, and the addition of nutrients and refiners such as active carbon can enhance the activity and species diversity of hydrocarbon pollutant

degrading bacteria in soil (Dadrasnia and Agamuthu, 2013; Tazangi et al., 2020; Karimpour et al., 2022).

Exhaustive and holistic investigation of the effect of petroleum wastes such as sludge on the environment-especially soil characteristics- is essential to develop suitable remediation methods. Thus, the present study aims to investigate the remedial effect of active carbon refiner on some physico-chemical characteristics of soil as well as hydrocarbon pollution removal in short-term.

## Materials and Methods

### General information on the experiments

Soil samples were collected from 0-30 cm depth within non-cultivated areas of the Shastkola region, Golestan province, Iran. Non-polluted control samples were passed from a 2 mm mesh to measure some physicochemical characteristics. Petroleum sludge was obtained from the sludge pit of the National Iranian Oil Products Distribution Company, Golestan province. The total petroleum hydrocarbons (TPHs) of the sludge were 643000 mg kg<sup>-1</sup> dry sludge. Petroleum sludge was air-dried and ground and then kept in black plastic bags until incorporation with soil. To prepare the petroleum sludge-polluted samples, soil and sludge were mixed with 70:30 ratios and the polluted soils were transferred into 2 kg pots to facilitate aeration. The pots were incubated at 28 °C and 50% field capacity (measured using the pressure plates method) for 30 days. The soils in the pots were mixed thoroughly every two weeks for better incorporation of soil and sludge (Chorom et al., 2010).

The experiment was conducted as a split plot in time based on a completely randomized design with three replicates over 60 days. Experimental treatment

included active carbon at 100 g and 0.3 mm size (W100S0.3), 100 g and 1.5 mm (W100S1.5), 100 g and 4 mm (W100S4), 150 g and 0.3 mm (W150S0.3), 150 g and 1.5 mm (W150S1.5), 150 g and 4 mm (W150S4) and control (C) (Table 1). NPK was added with a 10:2:1 (N:P:K) ratio as the nutrient to all treatments for the maintenance of C/N (10:1) (Teng, 2010). Active carbon was bought from Vazin Esfahan Company, Iran, and its properties are presented in Table 2. The pots were then kept at 28 °C and 50% field capacity for 60 days and were aerated every other day. pH, EC and TPH concentrations were measured every 10 days and organic carbon, nitrogen and C/N content measurement was done every 15 days until the end of the experiment. Aggregate stability was measured at the end of the experiment based on a completely randomized design.

The concentration of TPH in sludge-polluted soil after incubation was 544500 mg ka<sup>-1</sup>. After incubation, physicochemical characteristics of sludge and sludge-polluted soil were determined utilizing standard soil science methods. Soil texture was determined using the hydrometer method (Gee et al., 1986). Electrical conductivity and pH were measured in soil paste (1:2.5 ratio) (Jabson et al., 1972). Available phosphorous measurement was done using the Olsen method (Olsen et al., 1954). The Kjeldahl method was used to determine total nitrogen (Bremner et al., 1982). Organic carbon was measured using Walkley and Black (1934) method. Calcium carbonate content was determined via the back titration method using acid (Rowel, 1994) and potassium was measured utilizing the acetate ammonium extraction method through flame photometer apparatus (Table 2).

**Table 1.** Experimental treatments and their abbreviations

Treatment	Abbreviation
100 g weight and 0.3 mm size	W100S0.3
100 g weight and 1.5 mm size	W100S1.5
100 g weight and 4 mm size	W100S4
150 g weight and 0.3 mm size	W150S0.3
150 g weight and 1.5 mm size	W150S1.5
150 g weight and 4 mm size	W150S4
Carbon-free (control)	C

**Table 2.** Some physicochemical characteristics of soil, sludge and sludge-polluted soil

Soil properties	Unit	Non-polluted soil	Petroleum sludge	Polluted soil
EC	dS /m	3.70	9.16	9.39
pH	-	6.69	6.42	6.51
Organic carbon	%	1.08	53.04	39
K	mg / kg	140.53	90.34	100.38
Total N	%	0.44	0.77	.84
P	mg / kg	33.17	27.16	39.57
CaCO <sub>3</sub>	%	14.75	1	14.5
Field capacity (FC)	%	29.6	-	19.9
Texture	-	loam	-	-
Clay	%	20.5	-	-
Silt	%	46	-	-
Sand	%	33.5	-	-

**Table 3.** Properties of the active carbon used in the present study

Properties	Value	Properties	Value
Stability	>99%	Volume	950 mg/g
Particle size (mesh)	5×9 (0.3mm) 6×2 (1.5 mm) 3×2 (4mm)	Specific surface	>950 m <sup>2</sup> /g
Moisture	2% Max	Pore total volume	>1 g/cm <sup>3</sup>
Ash	5% Max	Density	0.44-0.48

### Measurement of total petroleum hydrocarbons (TPH)

In this method, the petroleum present in the soil is extracted using n-hexane solvent and transferred into the liquid phase. Two g of the polluted soil is mixed with 10 ml of n-hexane and the solution is put on the shaker at 150 rpm for 30 min. The solution is then centrifuged at 3000 rpm for 15 min. The supernatant is transferred into a tube and kept at room temperature. After this period, the weight of the remaining material in the tube was reported as TPH (mg kg<sup>-1</sup> soil) (Equation 1).

$$TPH \left( \frac{mg}{kg \text{ dry sludge}} \right) = \frac{\text{Weight difference of test tube (mg)}}{\text{Sample dry weight (gr)}} \times 1000 \quad (1)$$

### Nitrogen measurement

Nitrogen content was measured every 15 days using the Kjeldahl method. Five g of the polluted soil was placed in the Kjeldahl flask and 15 g catalyzer and 20 mL 97% H<sub>2</sub>SO<sub>4</sub> were added to the flask. The flask was kept at 380 °C for 1 hour. The sample became transparent after 1 hour and nitrogen was transformed into its mineral

form. Then, 250 ml of distilled water was added, and the samples were cooled at room temperature. The flask was then put in the distillation unit of the Kjeldahl apparatus, and the end of the distillation tube was placed inside the indicator. Distillation was continued until 100 to 150 mL of the solution was distilled. Then, the indicator was separated and titrated using 0.01 N H<sub>2</sub>SO<sub>4</sub> until its colour changed from green to red. The volume of the acid used was measured and the total nitrogen percentage was calculated using Equation 2 (Bremner et al., 1982).

$$\%N = \frac{(a-b) \times N \times 1.4 \times mcf}{S} \quad (2)$$

which a is H<sub>2</sub>SO<sub>4</sub> used for the sample (ml), b is H<sub>2</sub>SO<sub>4</sub> used for control (ml), S is the dry weight of soil (g), N is H<sub>2</sub>SO<sub>4</sub> normality and mcf is moisture correction factor  $\left( \frac{100 + \text{moisture} (\%)}{100} \right)$ .

### Organic carbon measurement

Organic carbon content was measured using Walkley and Black (1934) method. For this purpose, 0.05 g of ground soil was weighed in a 250 ml Erlenmeyer flask and 10 ml 1 N

potassium dichromate was added to the soil. Then, 20 ml  $\text{H}_2\text{SO}_4$  was added immediately to the soil solution, which was then shaken for a minute and kept still for 30 min. After cooling, 100 mL of distilled water was added to the Erlenmeyer flask. The ortho-phenanthroline indicator was added in the next step and titration was done following the addition of 1 N ferrous ammonium sulphate until the solution turned dark red. The volume of ferrous ammonium sulphate was recorded, and one soil-free sample was considered as a control. The organic carbon content of the soil was calculated using Equation 3.

$$\%C = \frac{(a-b) \times M \times 0.39}{s} \quad (3)$$

In which: a is the volume of ferrous ammonium sulphate used for the soil sample, b is the volume of ferrous ammonium sulphate used for control, S is the dry weight of soil (g) and M is the normality of ferrous ammonium sulphate.

#### pH and EC measurement

Ten g of dry samples were mixed with 25 mL distilled water and shaken for an hour, then the samples were kept still for 24 hours. Finally, pH and EC were measured using pH and EC meters. It must be noted that pH was reported after temperature correction at 25 °C (Jobson, 1972)

#### Soil structure stability measurement

The aggregate stability test was implemented using the wet sieving method and for this purpose, mesh series of 2, 1, 0.5 and 0.25 mm were used, and 50 of non-ground soil passed from a 4 mm mesh was placed on the largest mesh. The meshes were moved up and down with 1 cm movements at 45 rpm for 10 min. Then, the remaining aggregates on each mesh were washed and weighed after being dried. Also, correction related to sand content was done using Equation 4. Then, Equation 5 was used to calculate the mean weight diameter (MWD) of water-stable aggregates (Briedis et al., 2012).

$$w_i = \frac{w_{i(a+s)} - w_{i(s)}}{w_t - \sum_{i=0}^n w_{i(s)}} \quad (4)$$

which  $w_{i(a+s)}$  is aggregate mass along with sand and gravel in the given range,  $w_{i(s)}$  is

the mass of sand and gravel in the given size range,  $w_t$  is the total dry weight of soil and  $w_i$  is the weight ratio of the aggregates in the given range.

$$\text{MWD} = \sum_{i=1}^n w_i \bar{x}_i \quad (5)$$

In which  $\bar{x}_i$  is the arithmetic mean of aggregate diameter in each range. Also, the geometric mean diameter (GMD) of water-stable aggregates was calculated using Equation 6 (Marquez et al., 2004), in which  $w_i$  and  $x_i$  are the same as mentioned in Equation 5.

$$\text{GMD} = \exp[\sum_{i=1}^n w_i \log \bar{x}_i] \quad (6)$$

Mechanically dispersible clay (MDC), an index of soil microstructure instability, was measured using the Rengasamy method (Rengasamy et al., 1984). For this purpose, 50 g of the soil passed through a 2 mm mesh and 500 ml of distilled water was mixed to create a 1:10 suspension of water and soil, which was shaken at 65 rpm for an hour and then transferred into 1-litre cylinders. After 24 hours, the samples were shaken manually, and the temperature of the suspensions was recorded. After 3 hours and 50 minutes (according to the Stokes' law), sampling was done using a 25 mm pipette from a 5 cm depth of the suspension. The sample was weighed following being dried in the oven. Finally, MDC was calculated based on the total clay content of the soil.

#### Statistical analysis

Organic carbon, nitrogen, C/N ratio, pH, EC and TPH concentration in the polluted soil traits were carried out as a split-plot in time experiment based on a completely randomized design, whereas the data related to soil structure stability were analyzed as a completely randomized design. The analysis of variance was done using the SAS v.9.0 software. The least significant difference (LSD) method was used for the comparison of means.

#### Results and Discussion

The main effects of sampling times and active carbon on carbon and nitrogen content and C/N were significant at  $p < 0.01$

(Table 4). These also had a significant effect on pH, EC and TPH (Table 5) at  $p < 0.01$ . Also, the interaction of sampling

times and active carbon on organic carbon and nitrogen content (Table 5) and pH and TPH (Table 6) was significant at  $p < 0.01$ .

**Table 4.** Analysis of variance on the effect of active carbon sizes and weights on C, N and C/N over time.

SOV	df	MS		
		C	N	C/N
Active carbon	6	328.560**	0.484**	29.625 <sup>ns</sup>
Error I	14	2.815	0.023	11.636
Sampling time	3	1986.801**	2.271**	204.588**
Active carbon × Sampling time	18	8.701**	0.048**	1.787 <sup>ns</sup>
Error II	42	3.624	0.007	4.888
Total	83	---	---	---
CV (%)	---	8.92	8.28	11.03

ns and \*\* non-significant and significant at  $p < 0.01$ , respectively.

**Table 5.** Analysis of variance on the effect of active carbon sizes and weights on pH, EC and TPH over time.

SOV	df	MS		
		pH	EC	TPH
Active carbon	6	0.144**	1.521**	96870618481**
Error I	14	0.018	0.015	26825340.4
Sampling time	6	6.347**	5.775**	13168539116**
Active carbon × Sampling time	36	0.016**	0.033 <sup>ns</sup>	709744803.5**
Error II	42	0.005	0.006	42291099.77
Total	83	---	---	---
CV (%)	---	0.097	1.17	1.69

ns and \*\* denote non-significant and significant at  $p < 0.01$ , respectively.

The lowest content of organic carbon and nitrogen was observed in the W150S0.3 treatment, which decreased by 49.39 and 41.86% compared to control, respectively (Figure. 1a and 1b). Organic carbon and nitrogen content significantly decreased as active carbon weight and size increased and decreased, respectively. Apparently, porous structure and increased affinity of active carbon due to increased weight and decreased size of active carbon has led to enhanced degradation of carbon present in soil (Yeung, 2003; Yue, 2005), and microorganisms have used the soil nitrogen by degrading carbon to maintain their C/N ratio. Petroleum leakage leads to an increased C/N ratio, as crude petroleum is basically a mixture of carbon and hydrogen. This will result in N deficiency in petroleum polluted soil which delays the bacteria growth and exploitation of carbon resources unless the nitrogen required for

the degradation of petroleum hydrocarbons is added to the soil (Chorom et al., 2010).

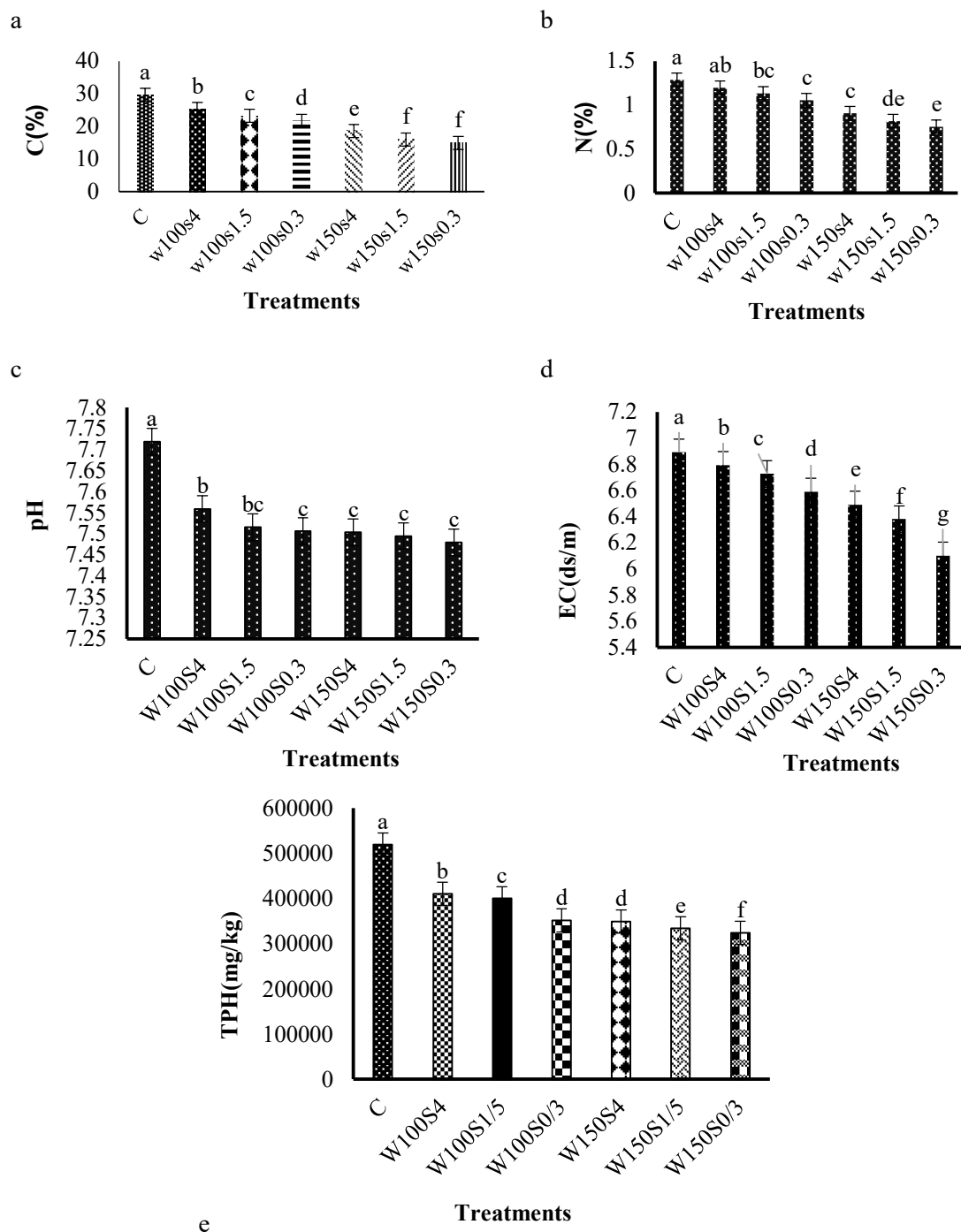
The highest values for pH and EC were obtained in control (7.72 and 6.89, respectively) and the lowest was recorded in W150S0.3 (7.47 and 6.1, respectively), which showed a 3.24 and 11.46% reduction compared with control. It must be noted that pH in W150S0.3 had no significant difference with W150S1.5, W150S4, W100S0.3 and W100S1.5. Overall, increased weights and decreased sizes of active carbon led to reduced pH and EC (Figure 1c and 1d).

Gradual reduction of pH indicates that microorganisms are involved in the biological degradation of hydrocarbons (Ughala et al., 2019). Crude petroleum contains acidic compounds such as naphthenic acid, stearic acid and valeric acid, and their degradation may lead to lower pHs in polluted soils (Ayoubi et al., 2020). Soil pH is an important factor in

biodegradation, as it affects the activity of heterotrophic microbes and alters the composition of the microbial community (Wick et al., 2011).

W150S0.3 treatment had the lowest TPH compared with the other treatments and showed 37.58 and 7.63% decrease

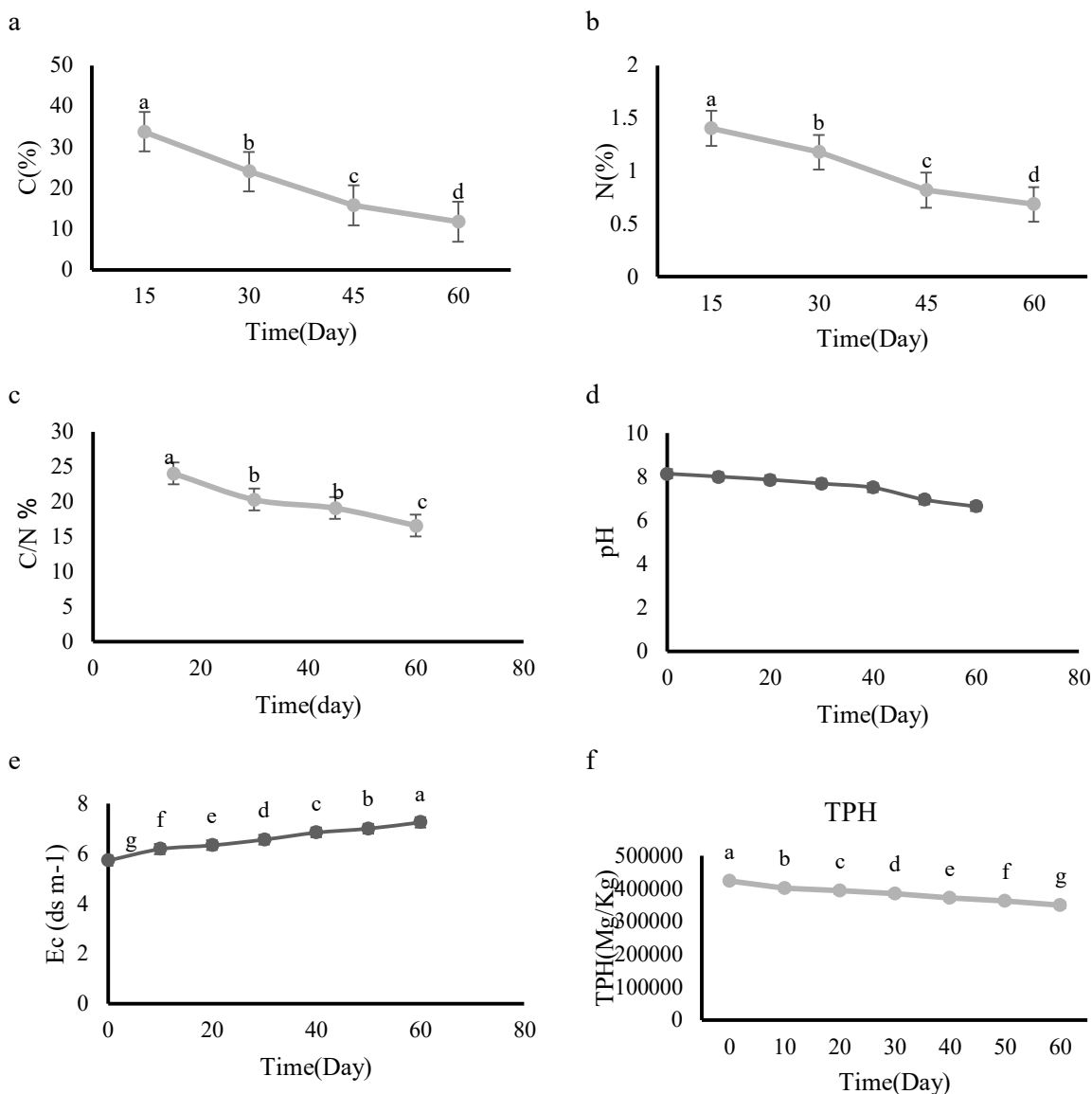
compared with the control and W100S0.3 treatments, respectively (Figure 1e). Active carbon irreversibly renders the hydrocarbon less dynamic and makes TPH available for biodegradation by limiting the volatility and leaching of hydrocarbons (Meynet et al., 2012; Brown et al., 2017).



**Figure 1.** The effect of active carbon treatments on carbon (a), nitrogen (b), pH (c), EC (d) and TPH (e). Similar letters denote the lack of significant difference. W and S show active carbon weight and size, respectively. C stands for control.

According to Figure 2, organic carbon, nitrogen, C/N, pH and TPH significantly reduced over time. The presence of petroleum pollution leads to reduced pHs, which may be attributed to the accumulation of organic hydrogen in petroleum sludge (Laurent et al., 2012). The

electrical conductivity of soil also increased over time (Figure. 2e). The application of active carbon enhances the biodegradation rate and reduces the concentration of petroleum hydrocarbons in soil (Rhodes et al., 2008, 2010).



**Figure 2.** Organic carbon (a), nitrogen (b) C/N (c), pH (d), EC (e) and TPH (f) of the polluted soil over time. Similar letters denote the lack of significant difference.

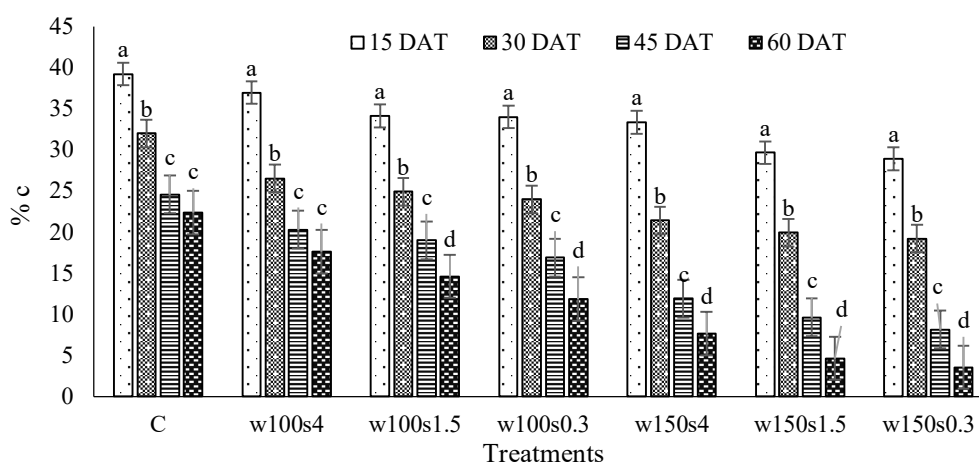
Microbial degradation capacity usually depends on the bioavailability of hydrocarbons as well as nutrient supply, aeration, temperature and pH (Wu et al., 2008). Therefore, the availability of nutrients required for degrading microorganisms—especially nitrogen—has led to increased

degradation of hydrocarbons, and TPH, organic carbon and nitrogen contents and C/N decrease over time (Figure 2). The absence of nutrients impedes petroleum hydrocarbon degradation and thus, C/N increases (Odokuma and Dickson, 2003).



The carbon content of the polluted soil significantly decreased over time. The highest decrease in organic carbon content was associated with W150S0.3, 60 days after treatment, which was 84.14% lower than the control. Increased weight and decreased size of active carbon led to the lower organic carbon content in all sampling time treatments. Carbon content in control, W100S4, W100S1.5, W100S0.3, W150S4, W150S1.5 and W150S0.3 treatments 60 days after treatment decreased by 42.95, 52.34, 57.25, 65, 76.99,

84.36 and 87.8% compared with those at 15 days after treatment, respectively (Figure 3). Decreased content of organic carbon may be attributed to the mineralization of organic matter and its degradation by microorganisms and the release of organic carbon in CO<sub>2</sub> form (He et al., 2018). In a study on the bioremediation of hydrocarbons in soil, it was reported that the carbon level of soil in all treatments was significantly reduced over time, which indicates the diminishing of carbon content due to microbial oxidation (Riffaldi et al., 2006).



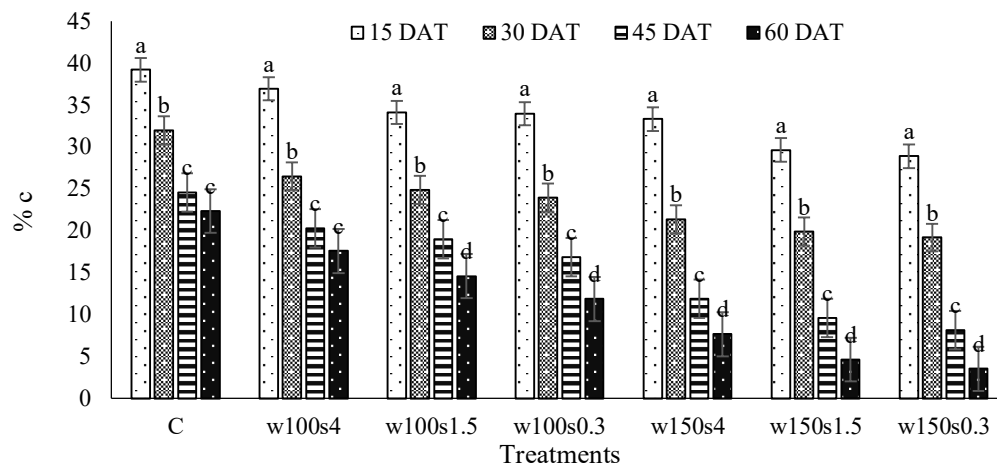
**Figure 3.** The effect of active carbon on the carbon content of the polluted soil over time. Similar letters in each set of columns denote the lack of significant difference. DAT: days after treatment. W and S show active carbon weight and size, respectively. C stands for control.

The nitrogen content of soil decreased with increasing days after treatment. The lowest content of soil nitrogen was recorded in W150S0.3 treatment 60 days after treatment which was 77.13% lower than the control at the same sampling time. Compared with the first sampling time, nitrogen content in control, W100S4, W100S1.5, W100S0.3, W150S4, W150S1.5 and W150S0.3 treatments 60 days after treatment decreased by 25.1, 30.55, 38.2, 47.5, 65.9, 75 and 80.6%, respectively. Therefore, increased weight and decreased size of active carbon led to lower nitrogen content in all sampling times (Figure 4).

Petroleum leakage leads to an imbalance in the C/N ratio, as crude petroleum is a mixture of hydrogen and carbon. This will lead to nitrogen deficiency in petroleum

polluted soils which may impede the growth of bacteria and thus, the degradation of petroleum hydrocarbons (Chorom, 2010). Therefore, it is necessary to add nutrients- especially nitrogen- to polluted soils to maintain the C/N ratio in microorganisms as well as to facilitate the rapid degradation of pollutants.

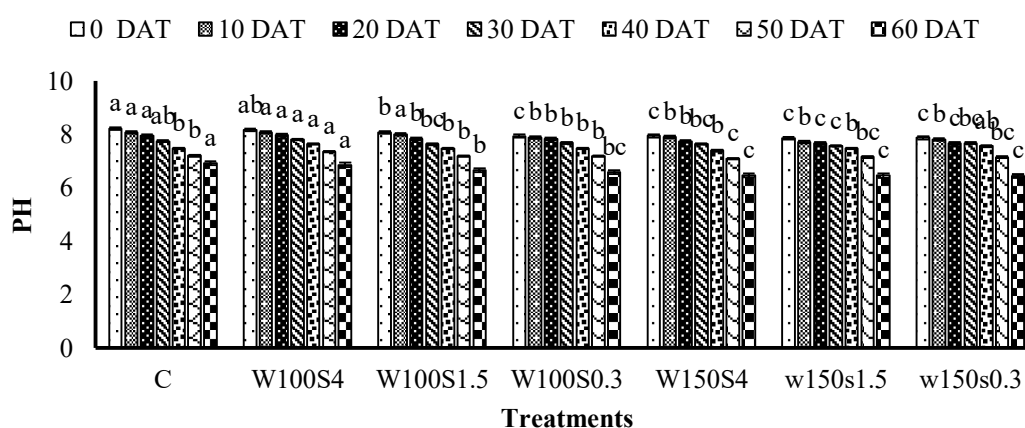
Hamoudi-Belarbi et al. (2018) reported that the addition of nutrients such as NPK in mineral form positively affects the bioremediation of petroleum pollution in soil. Fertilization will supply the hydrocarbonoclastic microorganisms with nitrogen and phosphorous and fastens their propagation. Nitrogen is essential for the growth and propagation of microbes responsible for TPH degradation (Yousefi et al., 2021).



**Figure 4.** The effect of active carbon on the nitrogen content of the polluted soil over time. Similar letters in each set of columns denote the lack of significant difference. DAT: days after treatment. W and S show active carbon weight and size, respectively. C stands for control.

In general, soil pH has decreased over time in all active carbon treatments (Figure. 5). In all sampling times, the highest and lowest pHs were observed in W100S4 and W150S0.3, respectively. The findings of Tang et al. (2012) showed that pH is reduced in petroleum polluted soil. Reduced pH may be due to the activity of TPH degrading bacteria which produce weak acids. The maximum activity of most microbial species occurs in a certain range

of pH, and the most suitable pH for rapid degradation of hydrocarbons is between 6.75 to 7.25 (Speight et al., 2012). Nwankwegu et al. (2016) monitored pH fluctuations in various bioremediation stages and attributed them to nutrient deficiency and accumulation of microbial metabolites. According to Roldan et al. (2012), the activity of microorganisms produces acidic compounds and thus, results in reduced pHs.



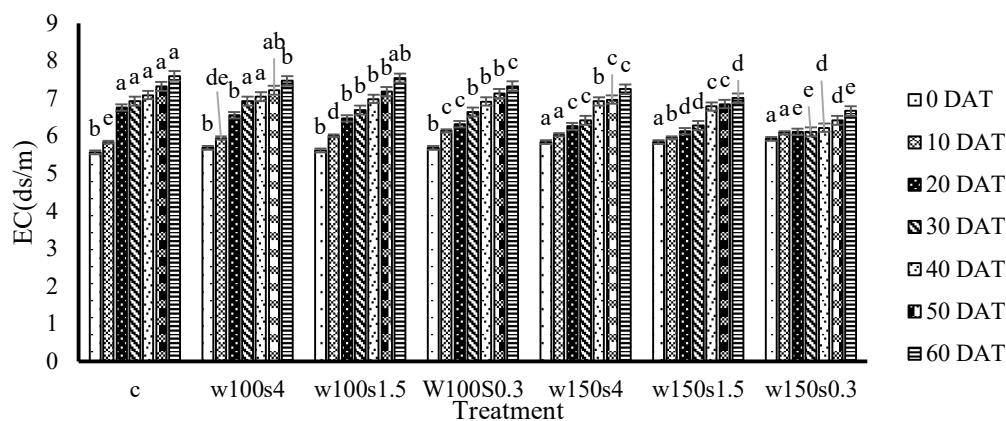
**Figure 5.** The effect of active carbon on pH of the polluted soil over time. Similar letters in each set of columns denote the lack of significant difference. DAT: days after treatment. W and S show active carbon weight and size, respectively. C stands for control.

The comparison of means related to the interaction effect of active carbon and sampling times (Figure 6) showed that soil EC decreased over time regardless of the

active carbon treatment. This may be due to the degradation of TPH, which in turn leads to the release of various mineral salts (He et al., 2018). Electrical conductivity in

control, W100S4, W100S1.5, W100S0.3, W150S4, W150S1.5 and W150S0.3 treatments 60 days after treatment increased by 22.1, 21.8, 22.52, 22.51, 22.62, 18.83 and 16.64%, respectively. The lowest increase in EC is related to 150 g active carbon weight and 0.3- and 1.5-mm sizes,

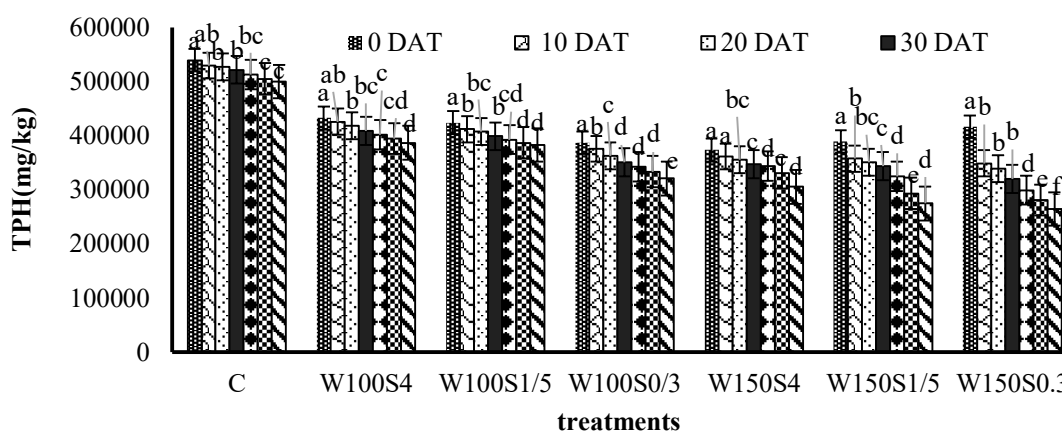
which may be due to the consumption of the nutrients released by hydrocarbon degradation. Increased salinity following petroleum pollution seemingly leads to the release of nutrients into soil solution (Balachandran et al., 2012).



**Figure 6.** The effect of active carbon on EC of the polluted soil over time. Similar letters in each set of columns denote the lack of significant difference. DAT: days after treatment. W and S show active carbon weight and size, respectively. C stands for control.

The concentration of TPH in all active treatments decreased over time (Figure 7), so that TPH in control, W100S4, W100S1.5, W100S0.3, W150S4, W150S1.5 and W150S0.3 treatments at the end of the experiment (60 days after treatment) respectively reduced by 7.33, 10.64, 9.79, 12.25, 12.69, 23.18 and 36.5%.

Therefore, increased weight and decreased sizes of active carbon have led to improved degradation of TPH in soil. The application of active carbon is demonstrated to accelerate the degradation of pollutants (Semenyuk et al., 2014). Another reason for enhanced degradation of TPH may be the role of active carbon in the soil as an adsorbent that adsorbs the pollutants. This optimizes the conditions for microorganism growth.



**Figure 7.** The effect of active carbon on TPH of the polluted soil over time. Similar letters in each set of columns denote the lack of significant difference. DAT: days after treatment. W and S show active carbon weight and size, respectively. C stands for control.

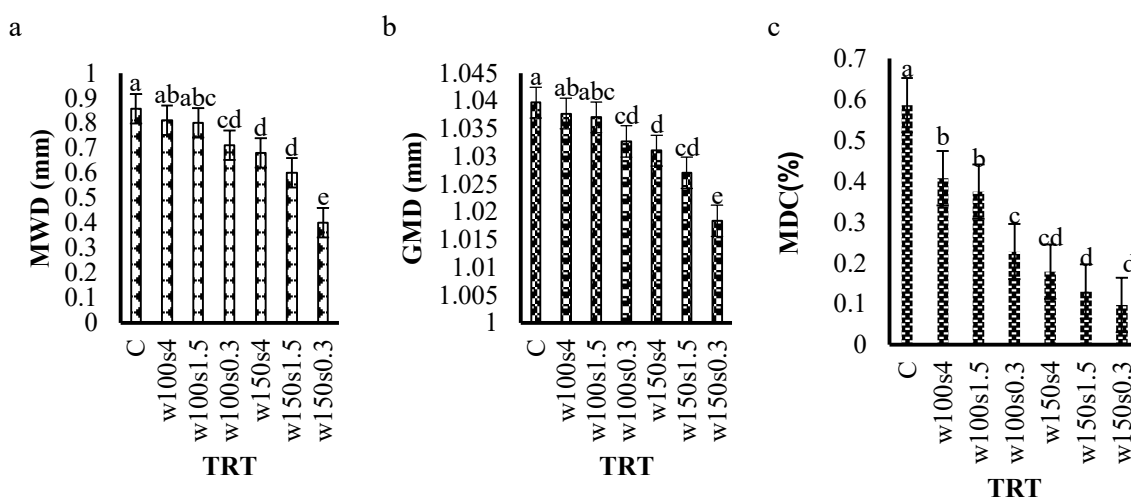
According to Table 6, the effect of active carbon on MWD, MDC and GMD was significant at  $p < 0.01$ . The highest and lowest MWDs were observed in control and W150S0.3, respectively, with the latter showing a 53% decrease compared with control (Figure 8a). It appears that increased active carbon weight has led to the reduction of MWD. Also, this increase in treatments containing smaller carbon sizes is more intense. The lowest GMD was obtained in the W150S0.3 treatment, which was 2% lower than the control (8b). Due to strong adsorption attributes resulting from a high specific area, fine porous structure and high affinity due to increased application of active carbon in W150S0.3, W150S1.5 and W150S4 treatments, more active carbon particles are adsorbed to petroleum hydrocarbons. Moreover, the hydrophobicity of petroleum hydrocarbon will probably decrease due to their

attenuation. Therefore, MWD and GMD will decrease in 150 g active carbon treatments during wet sieving, as the aggregates are passed to the lower meshes easier due to decreased hydrophobicity of aggregates (Mohammadi et al., 2021). The lowest and highest MDCs were observed in W150S0.3 and control treatments, respectively (Figure 8c). Therefore, increased weight and decreased size of active carbon have led to reduced MDC. The application of 0.3 mm active carbon as a high-affinity organic compound has resulted in the increased formation of a mineral-organic complex with clay. Therefore, in W150S4, W150S1.5 and W150S0.3 treatments, MDC against mechanical forces and water is reduced compared with control and 100 g carbon weight treatments (Brakorenko et al., 2016).

**Table 6.** Analysis of variance on the effect of active carbon sizes and weights on aggregate stability.

SOV	df	MS		
		MWD	GMD	MDC
Active carbon	6	0.073 **	0.000161 **	0.093 **
Error	14	0.005	0.00001	0.003
Total	20	---	---	---
CV (%)		9.78	0.31	17.88

ns and \*\* denote non-significant and significant at  $p < 0.01$ , respectively.



**Figure 8.** The effect of active carbon on MWD (a), GMD (b) and MDC (c). W and S show active carbon weight and size, respectively. C stands for control.

## Conclusion

The results of this study demonstrated the positive effects of increasing the weight and

decreasing the size of activated carbon on TPH degradation. The optimal treatment was activated carbon with a 0.3 mm particle

size and a weight of 150 g. The porous structure and high affinity of this treatment for TPHs created a favorable environment for the growth of TPH-degrading microorganisms by providing essential nutrients, oxygen, and moisture. Additionally, this treatment reduced the toxicity of TPHs, the hydrophobicity of aggregates, and the percentage of dispersible clay. As a result, aggregate stability improved, indicating enhanced soil structure and water retention in the polluted

soil. Electrical conductivity (EC) and pH, two important chemical properties, were also affected by the activated carbon treatment. The release of soluble salts and organic acids due to hydrocarbon degradation increased EC and lowered pH. These effects were observed over a 60-day period; however, long-term studies are needed to better understand the impact of refiners on the physicochemical characteristics of soil.

## References

- Achuba, F. I., and Peretiemmo-Clarke, B. O. 2008. Effect of spent engine oil on soil catalase and dehydrogenase activities. *International Agrophysics*. 22(1), 1-4.
- Agarry, S.E., Aremu, M.O., and Aworanti, O.A. 2013. Biodegradation of 2, 6-dichlorophenol wastewater in soil column reactor in the presence of pineapple peels-derived activated carbon, palm kernel oil and inorganic fertilizer. *Journal of Environmental Protection*. 4, 537-547.
- Agarry, S.E., Oghenejoboh, K.M., and Solomon, B.O. 2015. Kinetic modelling and half-life study of adsorptive bioremediation of soil artificially contaminated with bonny light crude oil. *Journal of Ecological Engineering*. 16(3), 1-13.
- Ayoubi, S., Samadi, M.J., Khademi, H., Shirvani, M., and Gyasi-Agyei, Y. 2020. Using magnetic susceptibility for predicting hydrocarbon pollution levels in a petroleum refinery compound in Isfahan Province, Iran. *Journal of Applied Geophysics*. 172, 103906 (In Persian).
- Azubuikwe, C.C., Chikere, C.B., and Okpokwasili, G.C. 2016. Bioremediation techniques—classification based on site of application: principles, advantages, limitations and prospects. *World Journal of Microbiology and Biotechnology*. 32(11), 1-18.
- Balachandran, C., Duraipandian, V., Balakrishna, K., and Ignacimuth, S. 2012. Petroleum and polycyclic aromatic hydrocarbons (PAHS) degradation and naphthalene metabolism in *Streptomyces* sp. (ERI-CPDA-1) isolated from oil contaminated soil. *Bioresource Technology*. 112, 83–90.
- Becker, B. R., de Souza, E. S., Martins, R. L., and da Luz Bueno, J. 2016. Bioremediation of oil-contaminated beach and restinga sediments using a slow-release fertilizer. *Clean (Weinh)*. 44(9), 1154-1162.
- Brakorenko, N. N., and Korotchenko, T.V. 2016. Impact of petroleum products on soil composition and physical-chemical properties. *IOP Conf. Ser. Earth Environmental Science*. 33, 012028.
- Bremner, J.M., and Mulvaney, C.S. 1982. Nitrogen-Total. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.) *Methods of soil analysis. Part 2. Chemical and microbiological properties*. American Society of Agronomy, Soil Science Society of America. Madison, Wisconsin, pp. 595-624.
- Brown, D. M., Okoro, S., van Gils, J., van Spanning, R., Bonte, M., Hutchings, T., Linden, O., Egbuche, U., Bruun, K.B., and Smith, J.W. 2017. Comparison of landfarming amendments to improve bioremediation of petroleum hydrocarbons in Niger Delta soils. *Science of The Total Environment*. 596, 284-292.
- Cai, Y., Wang, R., Rao, P., Wu, B., Yan, L., Hu, L., Park, S., Ryu, M., and Zhou, X. 2021. Bioremediation of petroleum hydrocarbons using acinetobacter sp. SCYY-5 isolated from contaminated oil sludge: Strategy and effectiveness study. *International Journal of Environmental Research and Public Health*. 18(2), 819.
- Chikere, C.B., Obieze, C.C., and Okerentugba, P. 2015. Molecular assessment of microbial species involved in the biodegradation of crude oil in saline Niger Delta sediments using bioreactors. *Journal of Bioremediation and Biodegradation*. 6(5), <http://dx.doi.org/10.4172/2155-6199.1000307>
- Chorom, M., Sharifi, H.S., and Motamedi, H. 2010. Bioremediation of a crude oil-polluted soil by application of fertilizers. *Journal of Environmental Health Science and Engineering*. 7(4), 319-326.
- Dadrasnia, A., and Agamuthu, P. 2013. Dynamics of diesel fuel degradation in contaminated soil using organic wastes. *International Journal of Environmental Science and Technology*. 10(4), 769-778.
- Das, S., Kuppunan, N., Channashettar, V.A., and Lal, B. 2018. Remediation of oily sludge-and oil-contaminated soil from petroleum industry: recent developments and future prospects. In: Adhya,

- T.K., Lal, B., Mohapatra, B., Paul, B., Das, S. (Eds.). Advances in Soil Microbiology: Recent Trends and Future Prospects. Springer, Singapore, pp. 165-177.
- Dexter, A. R., Richard, G., Arrouays, D., Czyż, E. A., Jolivet, C., and Duval, O. 2008. Complexed organic matter controls soil physical properties. *Geoderma*. 144(3-4), 620-627.
- Gee, G.W., and Bauder, J.W. 1986. Particle-Size Analysis. In: Klute, A. (Ed.) *Methods of Soil Analysis, Part 1. Physical and Mineralogical Methods*. American Society of Agronomy/Soil Science Society of America, Madison, USA, pp. 383-411.
- Hamoudi-Belarbi, L., Hamoudi, S., Belkacemi, K., Nouri, L., Bendifallah, L., and Khodja. M. 2018. Bioremediation of polluted soil sites with crude oil hydrocarbons using carrot peel waste. *Environment*. 5(11), 124.
- He, X., Zhang, Y., Shen, M., Zeng, G., Zhou, M., and Li, M. 2016. Effect of vermicomposting on concentration and speciation of heavy metals in sewage sludge with additive materials. *Bioresource Technology*. 218, 867-873.
- Hu, G., Li, J., and Zeng, G. 2013. Recent development in the treatment of oily sludge from petroleum industry: a review. *Journal of Hazard. Material*. 261, 470-490.
- Hutchinson, S. L., Banks, M.K., and Schwab, A.P. 2001. Phytoremediation of aged petroleum sludge. *J. Journal of Environmental Quality*. 30(2), 395-403.
- Jobson, A., Cook, F.D., and Westlake, D.W.S. 1972. Microbial utilization of crude oil. *Journal of Applied Microbiology*. 23(6), 1082-1089.
- Karimpoor, R., Ebrahimi, S., Malekzadeh, E., and Hassanpour-bourkheili. S. 2022. Bioremediation of total petroleum hydrocarbons in oil sludge-polluted soil using active carbon remediator *International Journal of Environmental Science Technology*. 1-12. <https://doi.org/10.1007/s13762-022-03964-9>
- Kriipsalu, M., Marques, M., and Maastik, A. 2008. Characterization of oily sludge from a wastewater treatment plant flocculation-flotation unit in a petroleum refinery and its treatment implications. *J. Mater. Cycles Waste Managment*. 10, 79-86.
- Kumari, S., Regar, R. K., and Manickam, N. 2018. Improved polycyclic aromatic hydrocarbon degradation in a crude oil by individual and a consortium of bacteria. *Bioresource Technology*. 254, 174-179.
- Laurent, F., Cebon, A., Schwartz, C., and Leyval, C. 2012. oxidation of a PAH polluted soil using modified fenton reaction in unsaturated condition affects biological and physic-chemical properties. *Chemosphere*. 86, 659-664.
- Liu, X., Yao, T., Lai, R., Xiu, J., Huang, L., Sun, S., Luo, Y., Song, Z., and Zhang, Z. 2019. Recovery of crude oil from oily sludge in an oilfield by sophorolipid. *Journal of Petroleum Science and Technology*. 37(13), 1582-1588.
- Marquez, C.O., Garcia, V.J., Cambardella, C.A., Schultz, R.C., and Isenhardt, T.M., 2004. Aggregate-size stability distribution and soil stability. *Soil Science Society of America Journal*. 68(3), 725-735.
- Meynet, P., Hale, S.E., Davenport, R.J., Cornelissen, G., Breedveld, G.D., and Werner, D. 2012. Effect of activated carbon amendment on bacterial community structure and functions in a PAH impacted urban soil. *Environmental Science and Technology Journal*. 46, 5057-5066.
- Mohammadi, N., and Khademalrasoul, A. 2021. Assessment of Zeoplant and biochar of sugarcane residual on mean weight diameter and Atterberg limits of soil contaminated with total petroleum hydrocarbon. *Iranian Journal of Soil and Water Research*. 52(2), 395-407 (In Persian).
- Neebee, E. 2020. Evaluation of Microbial Degradation of Crude Oil in a Polluted Tropical Soil. *International Journal of Advanced Science and Research*. 6, 169-179.
- Nwankwegu, A.S., Orji, M.U., and Onwosi, C.O. 2016. Studies on organic and in-organic biostimulants in bioremediation of diesel-contaminated arable soil. *Chemosphere*. 162, 148-156.
- Nwogu, T. P., Azubuike, C.C., and Ogugbue, C.J. 2015. Enhanced bioremediation of soil artificially contaminated with petroleum hydrocarbons after amendment with *Capra aegagrus hircus* (Goat) Manure. *Biotechnology Research International*. <https://doi.org/10.1155/2015/657349>
- Odokuma, L.O., and Dickson, A.A. 2003. Bioremediation of a crude oil polluted tropical rain forest soil. *Global Journal of Environmental Science and Management*. 2(1), 29-40.
- Olsen, S. R. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate (No. 939). US Department of Agriculture.
- Osuji, L. C., Idung, I. D., and Ojinnaka, C. M. 2006. Preliminary Investigation on Mgbede-20 Oil-Polluted Site in Niger Delta, Nigeria. *Chemistry and Biodiversity*. 3(5), 568-577.
- R. Riffaldi, Levi-Minzi, R., Cardelli, R., Palumbo, S., and Saviozzi, A. 2006. Soil biological activities in monitoring the bioremediation of diesel oil-contaminated soil. *Water Air Soil Pollution*. 170, 3-15.

- Raimondo, E. E., Aparicio, J.D., Bigliardo, A.L., Fuentes, M.S., and Benimeli, C.S. 2020. Enhanced bioremediation of lindane-contaminated soils through microbial bioaugmentation assisted by biostimulation with sugarcane filter cake. *Ecotoxicology and Environmental Safety*. 190, 110143.
- Rengasamy, P., Greene, R.S.B., Ford, G.W., and Mehanni, A.H. 1984. Identification of dispersive behaviour and the management of red-brown earths. *Soil Research*. 22(4), 413-431.
- Rhodes, A.H., Carlin, A., and Semple, K.T. 2008. Impact of black carbon in the extraction and mineralization of phenanthrene in soil. *Environmental Science Technology*. 42 (3), 740-745.
- Rhodes, A.H., McAllister, L.E., Chen, R., and Semple, K.T. 2010. Impact of activated charcoal on the mineralisation of <sup>14</sup>C-phenanthrene in soils. *Chemosphere*. 79 (4), 463-469.
- Roldán-Carrillo, T., Castorena-Cortés, G., Zapata-Peñasco, I., Reyes-Avila, J., and Olguín-Lora, P. 2012. Aerobic biodegradation of sludge with high hydrocarbon content generated by a Mexican natural gas processing facility. *Journal of Environmental Management*. 95, 93-98.
- Rowell, D.L., 1994. *Soil Science, Methods and Application*. Routledge, London.
- Saygılı, H., and Saygılı, G.A. 2019. Optimized preparation for bimodal porous carbon from lentil processing waste by microwave -assisted K<sub>2</sub>CO<sub>3</sub> activation: Spectroscopic characterization and dye decolorization activity. *Journal of Cleaner Production*. 226, 968 -976
- Semenyuk N. N., Yatsenko, V.S., Strijakova, E.R., Filonov, A.E., Petrikov, K.V., Zavgorodnyaya, Y.A., and Vasilyeva, G.K. 2014. Effect of activated charcoal on bioremediation of diesel fuel contaminated soil. *Microbiology*. 83(5), 589–598.
- Speight, J. G., and Arjoon, K.K., 2012. *Bioremediation of Petroleum and Petroleum Products*. John Wiley & Sons.
- Sui, X., Wang, X., Li, Y., and Ji, H. 2021. Remediation of petroleum-contaminated soils with microbial and microbial combined methods: Advances, mechanisms, and challenges. *Sustainability*. 13(16), 9267.
- Tarno, H., Qi, H., Endoh, R., Kobayashi, M., Goto, H., and Futai, K. 2011. Types of frass produced by the ambrosia beetle *Platypus quercivorus* during gallery construction, and host suitability of five tree species for the beetle. *Journal of Forestry Research*. 16(1), 68-75.
- Tazangi, M. H., Ebrahimi, S., Nasrabadi, R.G., and Naeeni, S.A.M. 2020. Kinetic monitoring of bioremediators for biodegradation of gasoil-polluted soil. *Water Air Soil Pollution*. 231, 418.
- Ughala, E., Ogugbue, C.J., and Okpokwasili, G.C. 2019. Enhanced remediation of a long term crude oil polluted soil using soya bean and poultry residues. *Int. J. Environ. Res. Public Health*. 6(8), 210-218.
- Vickers, N. J., 2017. Animal communication: when i'm calling you, will you answer too?. *Current Biology* 27(14), 713-715.
- Wick, A.F., Haus, N.W., Sukkariyah, B.F., Haering, K.C., and Daniels, W.L. 2011. *Remediation of PAH-Contaminated Soils and Sediments: A Literature Review*, CSES Department, Internal Research Document, Blacksburg, VA, USA.
- Wu, Y., Luo, Y., Zou, D., Ni, J., Liu, W., Teng, Y., and Li, Z. 2008. Bioremediation of polycyclic aromatic hydrocarbons contaminated soil with *Monilinia* sp.: Degradation and microbial community analysis. *Biodegradation*. 19, 247 -257.
- Xin, L. I., Hui-hui, Z. H. A. N. G., Bing-bing, Y. U. E., Nan, X. U., Wen-xu, Z. H. U., Ju-wei, H. U., and Guang-yu, S. U. N. 2012. A Effects of *Festuca arundinacea* on the microbial community in crude oil-contaminated saline-alkaline soil. *Chinese Journal of Applied Ecology*. 23(12), 3414–3420.
- Yeung, P.Y., Johnson, R.L., and Xu, J.G. 1997. Biodegradation of Petroleum Hydrocarbons in Soil as Affected by Heating and Forced Aeration. *Journal of Environmental Quality*. 26, 1511-1516.
- Yousefi, K., Mohebbi, A., and Pichtel, J. 2021. Biodegradation of Weathered Petroleum Hydrocarbons Using Organic Waste Amendments. *Applied and Environmental Soil Science*. <https://doi.org/10.1155/2021/6620294>
- Yue, Z., and Economy, J. 2005. Nanoparticle and nanoporous carbon adsorbents for removal of trace organic contaminants from water. *Journal of Nanoparticle Research*. 7(4), 477-487
- Zhang, L., Gu, H., Sun, H., Cao, F., Chen, Y., and Chen, G.Z. 2018. Molecular level one -step activation of agar to activated carbon for high performance supercapacitors. *Carbon*. 132, 573 -579.

