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

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Article Info	Abstract
	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.

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; Karimpoor 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^{-1} 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^{-1}. 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$_3$	%	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^2/g
Moisture	2% Max	Pore total volume	>1 g/cm^3
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^{-1} soil) (Equation 1).

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

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$_2$SO$_4$ 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$_2$SO$_4$ 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$_2$SO$_4$ used for the sample (ml), b is H$_2$SO$_4$ used for control (ml), S is the dry weight of soil (g), N is H$_2$SO$_4$ normality and mcf is moisture correction factor ($\frac{100+moisture\ (\%)}{100}$).

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 H_2SO_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.

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

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).

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

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.

$$MWD = \sum_{i=1}^{n} w_i \bar{x}_i \tag{5}$$

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.

$$GMD = \exp\left[\sum_{i=1}^{n} w_i \log \bar{x}_i\right] \tag{6}$$

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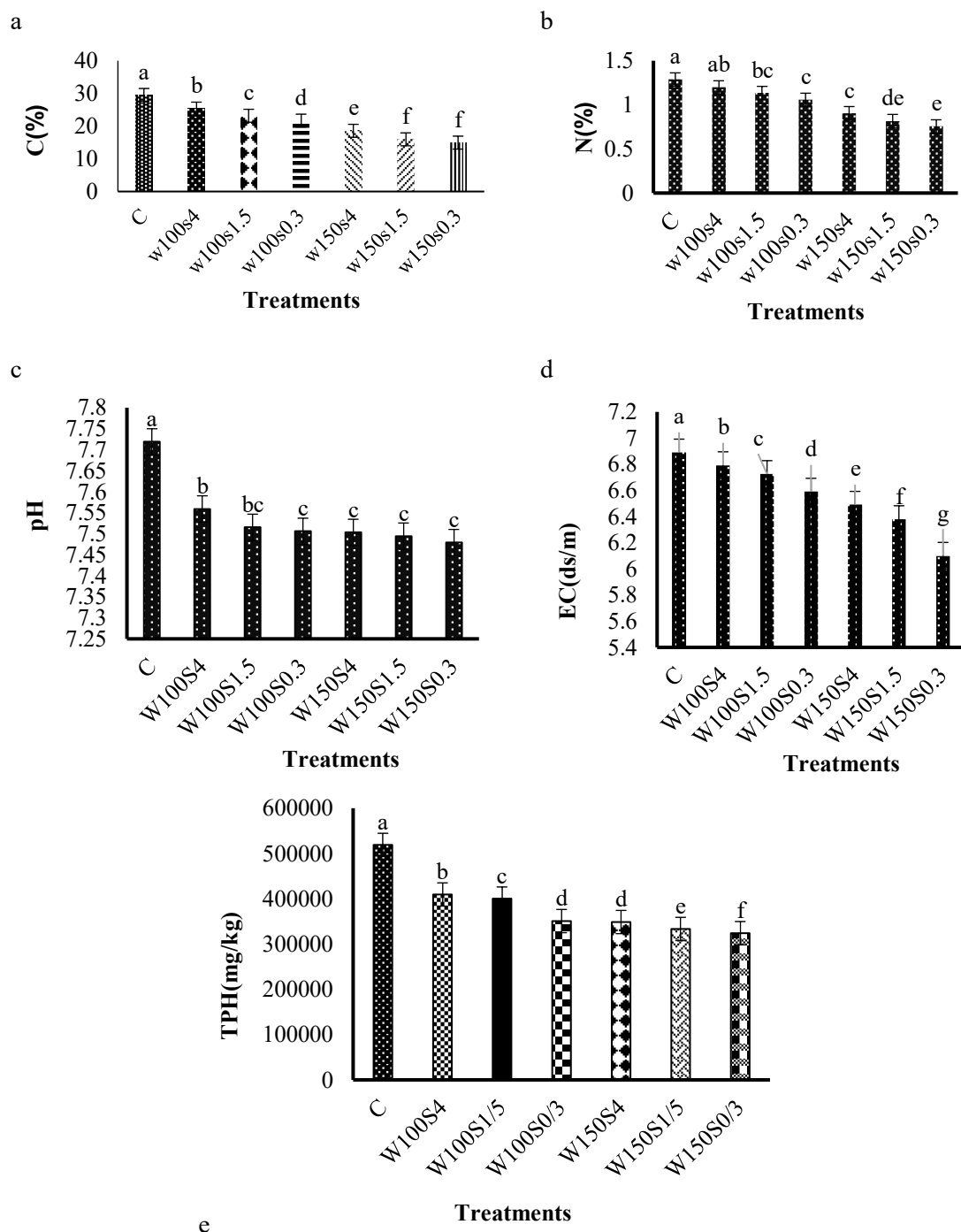
Figure 1. The effect of active carbon treatments on carbon (a), nitrogen (b), pH (c), EC (d) and TPH (e). Similar letters denote the lack of significant difference. W and S show active carbon weight and size, respectively. C stands for control.

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).
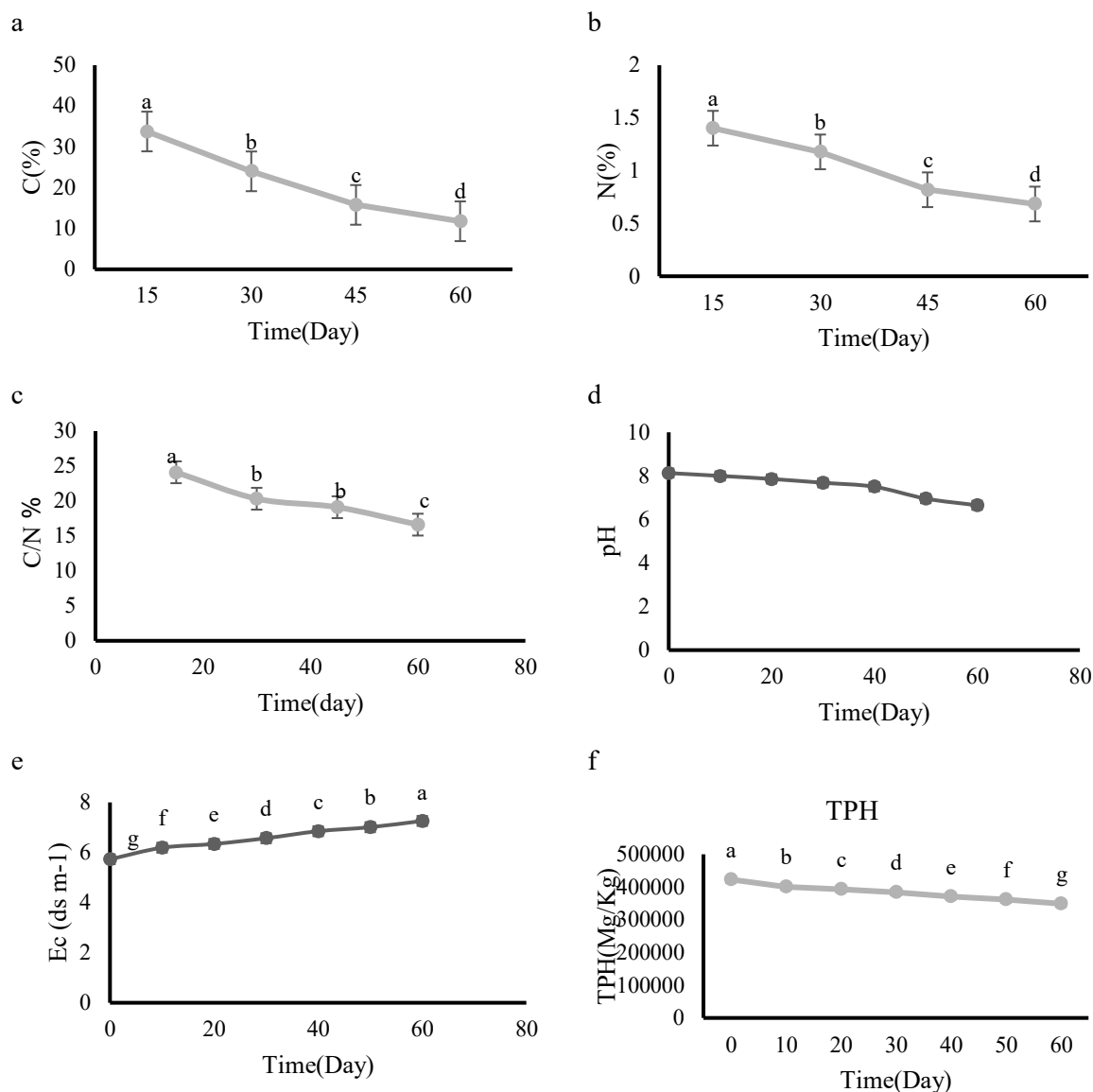

Figure 2. Organic carbon (a), nitrogen (b) C/N (c), pH (d), EC (e) and TPH (f) of the polluted soil over time. Similar letters denote the lack of significant difference.

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_2 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).
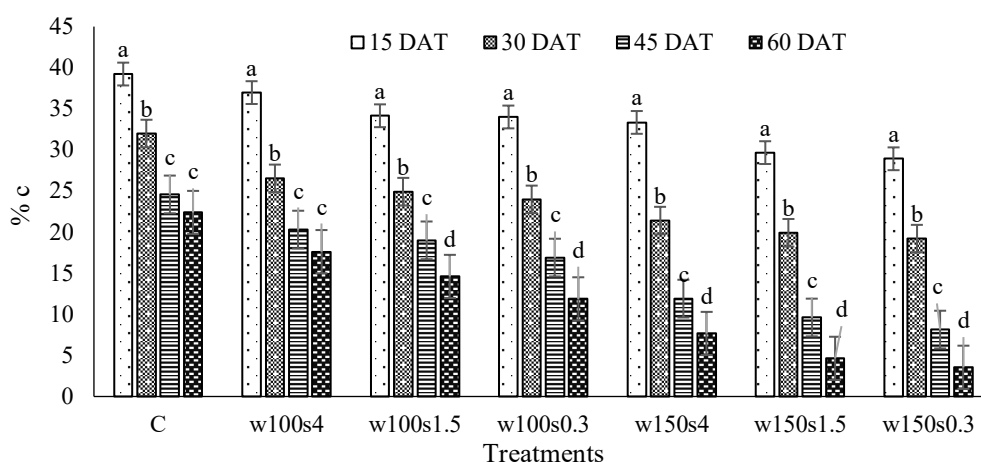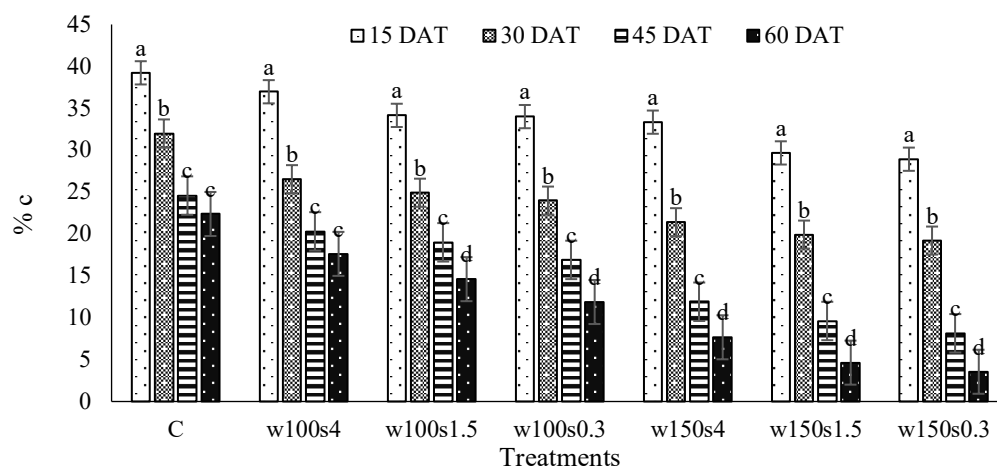

Figure 3. The effect of active carbon on the carbon content of the polluted soil over time. Similar letters in each set of columns denote the lack of significant difference. DAT: days after treatment. W and S show active carbon weight and size, respectively. C stands for control.

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.
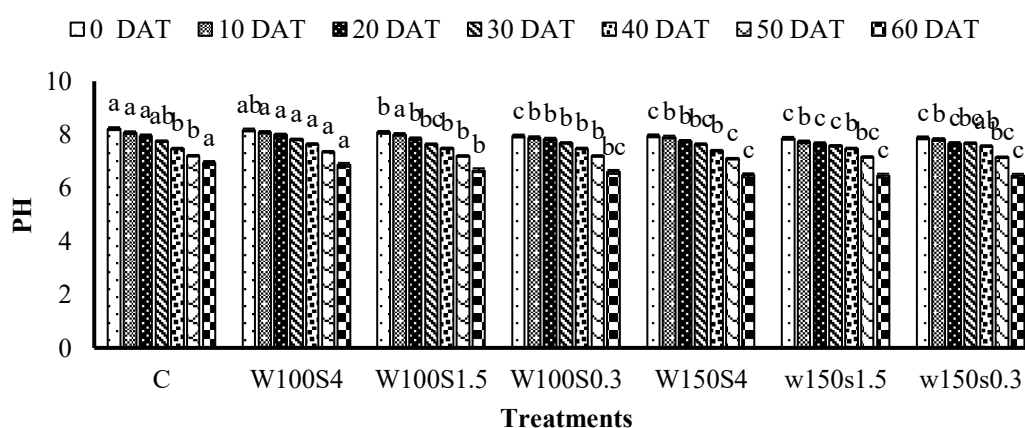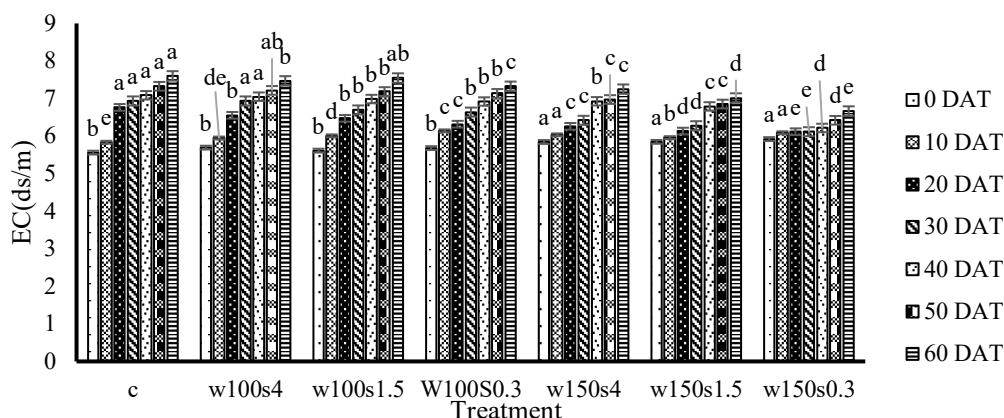

Figure 5. The effect of active carbon on pH of the polluted soil over time. Similar letters in each set of columns denote the lack of significant difference. DAT: days after treatment. W and S show active carbon weight and size, respectively. C stands for control.

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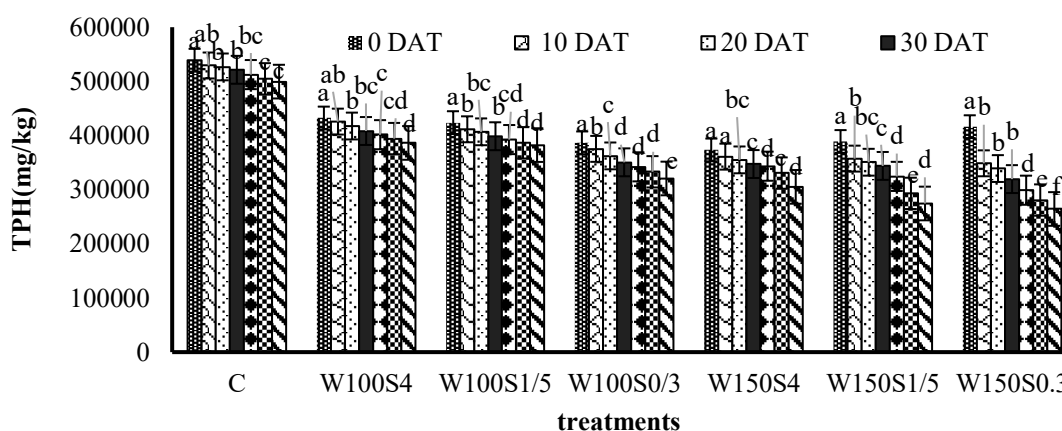
Figure 7. The effect of active carbon on TPH of the polluted soil over time. Similar letters in each set of columns denote the lack of significant difference. DAT: days after treatment. W and S show active carbon weight and size, respectively. C stands for control.

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.

a

b

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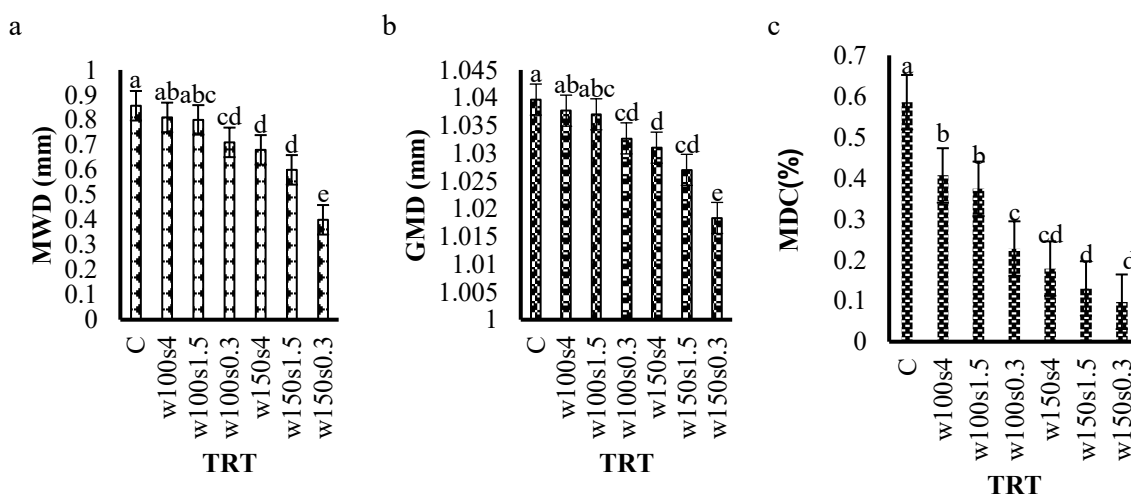
Figure 8. The effect of active carbon on MWD (a), GMD (b) and MDC (c). W and S show active carbon weight and size, respectively. C stands for control.

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.

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