

Original Research Article

Impact of Oil Field Effluent on Soil Health and Physiological Growth in Small Tea Farms of Shalmari OCS-1, Dibrugarh, Assam

Comment [DK1]: Language of whole manuscript should be Times New Roman and font size 12 except title of article (which should be 14).

Formatted: Right

Comment [DK2]: Title should be as Capital each word (As per Journal format)

Abstract

Aims: The investigation was undertaken to study the impact of spillage in the tea plantations in nearby oil fields with probable effect on the soil health and the growth of the tea crop with the following objectives to study the released from the oil fields on the physiology of tea crop and to study the impact of effluent on soil physicochemical in tea plants.

Study design: Analysis of variance (ANOVA) for two factorial RBD was carried out using OPSTAT.

Place and Duration of Study: The field study was carried out in Shalmari No. 1 near the Tingkhong tea estate of Dibrugarh. The laboratory works were carried out in the Department of Tea Husbandry & Technology and Department of Soil Science, Assam Agricultural University, Jorhat, Assam, between March'19 to February'20.

Methodology: Two tea-growing seasons viz. Rainflush and autumn flush were considered. Samples were collected in four distances at an interval of 0-21 m, 21-42 m, and 42-63 m and beyond 63m (control site). A total of 24 plant samples was collected for both the season with respect to 4 distance and 3 replication. In the case of soil samples, a total of 24 soil samples were collected for both the season with respect to 4 distance and 3 replication.

Results: Plant parameters like water saturation deficit increased in crude oil affected site while decreased the relative turgidity, stomatal count, in the tea plants grown in the contaminated site. Bulk density, pH, organic carbon, and available nutrients in the contaminated site of the soils contaminated site was increased. However, porosity, hydraulic conductivity and electrical conductivity was/were low in the contaminated area.

Conclusion: The study reveals oil effluent spillage as a major factor for plant growth and soil quality deterioration and the impact was more pronounced in the vicinity of the drilling point adversely affecting plant physiological, soil p physico-chemical parameters.

1. INTRODUCTION

Tea (*Camellia sinensis*) is an evergreen subtropical plant native to Asia, but presently tea is grown around the world. The tea plant is considered the most important crop in Assam as the tea industry of Assam is the single largest one in the state playing a dominant role in the economy of the state. Assam is also the oldest oil-producing state in India and a great contributor to crude oil and natural gas in the national economy.

Tea and crude oil drilling industries in Assam started in the eighteenth century and are vital for the economic perspective of the state. The occurrence of several crude oil drilling sites is in and around tea plantations, which is very common, especially in upper Assam. Assam is also the oldest oil-producing state in India and a great contributor to crude oil and natural gas in the national economy. The petroleum industry of Assam has a significant role in the process of industrialization in the State. Tea and crude oil drilling industries in Assam, India started in the eighteenth century. Both these two industries are vital from the socio-economic perspective of the state. In upper Assam, both these two industries are adjacent to each other as both the industries play the backbone of the state

Tea being a perennial crop, soil plays a vital role in the growth and production of tea. Many a time oil effluent from the oil drilling sites affects the tea field of nearby areas by contaminating the soil and so the production. This oil spill/effluent includes spill of crude oil or oil distilled products that can pollute the surface of the land, air, and water environments. Oil Spill may lead to degrading effects on the environments and living organisms, including humans, due to the discharge of various organic compounds that make up crude oil and oil distillate products, the majority of which include various individual hydrocarbons.

Crude oil affects the soil's physical and biological properties and reduces the growth and resistance of the plants to biotic and abiotic factors thus making them more vulnerable to pathogen infestation [1;2] Crude oil contamination is severely affecting the tea industry in Assam, as it reduces the production and quality of the produced tea. various physical, chemical, and thermal management practices are the common techniques to manage the crude oil-contaminated sites.

One of the most prospective techniques for the restoration of oil-contaminated soils is environmentally friendly, cost-effective phytoremediation. Phyto-remediation by planting *Calamagrostis angustifolia*, *Cyperus brevifolius* etc. has the potential to simultaneously restore and remediate the petroleum hydrocarbon-contaminated wetlands [3;4].

Currently with the study of various works done by various researchers on oil spillage affecting the tea industry of Assam it was observed that the small growers owning tea plantations near the oil industry were facing several cultivation problems as well as economic problems which were gradually resisting their income generation. In this study plant, physiological parameters, and soil physical and chemical parameters have experimented. Therefore, this research work has been taken to study the impact of spillage in the tea plantations in nearby oil fields with probable effect on soil health and the growth of the tea crop.

Formatted: Space After: 12 pt

2. MATERIAL AND METHODOLOGY

Experimental site

For the present investigation, the field study was carried out in the village Shalmari No. 1 near Tingkhong tea estate of Dibrugarh district (27.2648° N lat; 95.1354° E long), situated in the eastern part of Assam under the Agro-climatic zone of Upper Brahmaputra Valley zone of Assam. The site selected is nearer to Oil Collecting Station (OCS) number 2 with well number 17, 25, 30, 44, and oil exploring activities are carried out by Oil India Limited (OIL).

The laboratory works were carried out in the Department of Tea Husbandry & Technology and Department of Soil Science, Assam Agricultural University, Jorhat, Assam.

2.1 Collection and analysis of plant samples

Samples for the plant were carried out in two tea growing seasons viz. Rainflush, and autumn flush with four distances. In each season, twelve samples were collected at an interval of 0-21 m (D1), 21-42 m (D2), and 42-63 m (D3) and beyond 63m (Dc) (control site) starting from the effluent spilling point for the affected plot. Thirty-five numbers of bushes were selected (7x5) for each distance, and within these bushes, leaves were collected. For one season, a total of 12 samples were collected. A total of 24 samples were collected in both the season with respect to four distances and within each distances three replication were used in the entire investigation period. Sampling was done manually and was carried with PP bags.

2.1.1 PLANT PHYSIOLOGICAL PARAMETERS

2.1.1.1. Relative water content

The relative turgidity technique was determined by [Weatherley](#) [5] The technique consists essentially in comparing the water content of leaf tissue when freshly sampled with the fully turgid water content and expressing the result on a percentage basis, formula. Circular discs of 2cm diameter each were punched with the help of a cork borer of similar diameter from the fifth leaf of the tea clones. Care was taken to avoid the main veins. Fresh weight for each leaf disc was taken, and they were then floated in distilled water and stored at room temperature for 24 hours, drying up the surface moisture of the discs with the help of a blotting paper, the turgid weight of each disc was recorded. Then the discs were oven-dried at 80 C for 12 hours following which the oven-dry weight was recorded [6]

2.1.1.2 Water saturation deficit

The water saturation deficit and relative turgidity were determined by the disk method [7]. The water saturation deficit was determined by the disk method with extrapolation of saturation curves: disks of 8 mm. in diameter were punched out of the experimental leaves.

2.1.1.3 Stomatal count

The estimation of the number of stomatal was determined by the method given by [Beakbane and Majumdar](#) [8]. The number of stomata was measured in the mid position of the lower leaf surface, taking the help of the impression methods.

2.1.1.4 Leaf area

The leaf area measurement was conducted by the Millimeter graph paper method. In this method, a leaf is taken and traced over graph paper, and the grids covered by the leaf are counted to give the area. [9]

2.1.1.5 Specific leaf weight

Specific leaf weight is calculated by dividing the dry weight of leaves by their surface area [10].

2.2 Collection and analysis of soil samples

2.2.1 SOIL PHYSICAL PARAMETERS

Table 1: Methodology for soil parameters

Soil parameters	Unit	Method	Reference
Bulk density	mg m ⁻³	gravimetric method using undisturbed soil cores	[11]
Porosity	%	Keen Raczkowski box method	-
Hydraulic conductivity	cm m ⁻¹	Core sampler method	[12]
Soil particle analysis	%	pipette method	[12]

Comment [DK3]: May be re-written in text form.

2.2.2 SOIL CHEMICAL PARAMETERS

Table 2: Methodology for soil parameters

Soil parameters	Unit	Method	Reference
pH	mg m ⁻³	pH meter	[13]
Organic carbon	g kg ⁻¹	Walkley and black's titration	[14]
Electrical conductivity	dS m ⁻¹	measured at a soil: water ratio of 1:2.5 by the help of EC meter	[15]
Available nitrogen	kg ha ⁻¹	Kjeldahl's method (alkaline potassium permanganate)	[16]
Available phosphorous	kg ha ⁻¹	Bray's method	[13]
Available potassium	kg ha ⁻¹	Flame Photometric method	[13]

2.3 Statistical analysis

Analysis of variance (ANOVA) for two factorial RBD was carried out using OPSTAT and the comparison of means was done by calculating critical difference (CD) at a 5% probability level. The correlation was carried out using the IBM Statistical Package for Social Science (SPSS), version 18.

3. RESULTS AND DISCUSSION

3.1 Plant parameters

3.1.1 Physiological parameters

3.1.1.1 Relative turgidity

From the present experiment, it was observed that the relative turgidity content of the experimental tea samples varied significantly for both distance and season from the oil effluent point. Among the distances, DC and D3 were highly significant. During the rainy season (S1) the relative turgidity was recorded highest in the distance DC (beyond 63m) with relative turgidity content 87.3% whereas the lowest relative turgidity was recorded in distance D1 (0-21m) with relative turgidity content 83.13% (table 3; figure 1.1). During the autumn season (S2) the relative turgidity was recorded highest in the distance DC (beyond 63m) with relative turgidity content 91.04% whereas the lowest relative turgidity was recorded in distance D1 (0-21m) with relative turgidity content 81.15% (table 3, figure 1.1).

-This may be explained as there was a reduction in water content and a high water deficit in the experimental tea plants of the contaminated sites. The results are in conformity with the [17] He observed that due to petroleum contamination, there was a reduction in the water content in the soil leading to low leaf relative water content of *A. fruticosa* seedlings. He also reported that due to the effect of petroleum contamination in soil on the water status which can be attributed to the hydrophobic properties of petroleum which have altered water infiltration and humidity of the soil [18; 19]). Similar results were reported by [20] Due to this uptake of water from the soil becomes difficult for the plants leading to a deficit in water and minerals content in the body. This increase in relative turgidity content with increased distance was possibly due to the presence of more amount hydrocarbons near the spillage point, which led to the blockage of pore spaces with oil particles [21] reported that there was a significant reduction in relative water content of *Vigna unguiculata* in crude oil polluted site which indicate inadequate water for plant nourishment and turgidity and subsequent wilting, significant-high water deficit from the polluted soil.

When seasons were considered, the minimum relative turgidity content was recorded in the rainy season than the autumn season. Seasonal variation in relative turgidity content may be due to the amount of precipitation. Due to more precipitation in the rainy season leaves tends to accumulate more amount of water as compared to the autumn season

3.1.1.2 Water saturation deficit

The water saturation deficit of the experimental tea samples varied significantly with respect to distances and seasons. Among the distances, D1 showed the maximum Water saturation deficit in both rainy and autumn seasons (16.89% and 18.5% respectively and DC showed minimum Water saturation deficit in the both rainy and autumn season (12.7% and 8.75% respectively) presented in (Table 3, figure 1.2). However, among the distances, D3 and DC the water saturation deficit was highly significant.

A possible reason for the increase in water deficit near the effluent site may be due to the uptake of oil components by ~~the~~ plant via roots, stems, and accumulate in the leaves. The oil contains toxic components, which may alter the integrity and permeability of plant membranes leading to disturbance of both carbon metabolism and ion in the leaves and

Comment [DK4]: 1. Merge two to three graph in one. Reduce number of graphs. Similarly, reduce the number of Tables and paste in landscape page.

2. Optionally, less important data may be given in supplementary file and only more useful data be presented in this manuscript. Accordingly, text of data presented in supplementary file may be deleted from this manuscript.

3. Discussion is well written. Though, concise it as it is too lengthy.

Formatted: Space After: 12 pt

water uptake in the roots [22;23;24] A similar increase in water deficit in the crude oil-contaminated site was reported by [21]. They confirmed that due to a reduction in the water content in the plants there was high water deficit in the polluted site. Furthermore, it cannot be neglected that the hydrophobic nature of petroleum hydrocarbons prevents water from spreading inhomogeneous in the contaminated soil, resulting in a water deficiency [25;18;26]

3.1.1.3 Stomata count

From the present experiment, it was observed that the stomatal count of the experimental tea samples varied significantly concerning distance from the oil effluent point. When seasons were considered, then there was no significant difference observed in the number of stomatal counts. Among the distances, DC and D2 were highly significant. Between the distances DC (showed the maximum number of stomatal count in both rainy and autumn season, i.e. 20.33 and 23 respectively while the minimum number of the stomatal count was observed in the distance D1 i.e. 18.67 and 16.67 for both rainy and autumn season respectively. Presented in (Table 3, figure 1.3)

The results are in conformity with [27], who observed that stomata in *Chromolaena odorata* were grossly affected by crude oil, which manifested as distortion and reduction in the number of stomata per unit area of the leaf. Also, similar results were reported by [28] in their experiment with *Cyperus brevifolius* (Rottb.)

Transpiration rate (T_r) is closely related to stomatal conductance, and the decline of the latter is always accompanied by that of the former, in other words, the two make consonant changes because stomatal conductance is the main mechanism of regulating transpiration [29] Thus, a conclusion can be drawn that the decline in photosynthetic rate results from the decreasing photosynthetic activity of mesophyll cells, the stomatal conductance with the high petroleum concentrations has a dramatic fall. The intercellular CO_2 concentration is also in decline and reaches a significant level under the petroleum concentration, from which a conclusion can be drawn that the decline in photosynthetic rate is attributed to that in stomatal conductance.

3.1.1.4 Leaf area

Leaf area measurement for experimental tea samples both for control and contaminated site showed a significant difference between distances and seasons. Among the distances, DC and D2 were highly significant. Between the distances, DC showed the highest Leaf area for both rainy and autumn seasons (34.02 cm^2 and 33.71 cm^2 respectively (table 4, figure 2.1). While D1 showed the lowest leaf area for both the rainy and autumn season (32.28 cm^2 and 30.84 cm^2 respectively (table 4, figure 2.1). It was observed that the Leaf area was highest in the rainy season than the autumn season in all the distances

The findings were in agreement with [30] who reported that contamination of soil with crude oil showed significant decreases in leaf area. Similar results were reported by [28] that there was a gradual reduction of leaf size in all the crude oil treatments as compared to control due to a gradual increase in crude oil concentration. [31] discussed that crude oil caused a reduction of leaf size in *E. crassipes*, due to morpho-anatomical modifications, irregular distribution of the aerenchyma air spaces, or even its absence. He explained that the toxicity of heavy metal ions present in crude oil is due chiefly to their interference with electron transport in respiration and photosynthesis, and the inactivation of vital enzymes. As a result, the energy status is lowered, and the uptake of mineral nutrients decreases, reducing growth [32]

When seasons were considered, the autumn season showed less leaf area than the rainy season. This variation might be due to less amount of precipitation during the autumn season as compared to the rainy season. During the rainy season, more amount of water tends to accumulate in tea leaves, making the leaves more turgid.

3.1.1.5 Specific leaf weight

It was observed that the specific leaf weight of the tea leaf samples varied significantly in different seasons. It was found that the Leaf weight of the experimental plants increased during the rainy season than the autumn season from 7.7 to 7.2 mg cm⁻² respectively (table 4, figure 2.2). Specific leaf weight in the oil effluent contaminated site did not show any significant variation with respect to distance

The possible reason for the decrease of leaf weight during the autumn season may arise due to less precipitation during the autumn season. Also, a drought stress condition could have occurred because of oil contamination. This could be due to the hydrophobic coating of oil around the soil particles, causing a reduction in water uptake also results in low relative water content.

Table 3: Plant physiological parameters (i)

Season distance	Relative turgidity(%)			Water saturation deficit(%)			Stomatal count(in numbers)		
	Rainy season	Autumn season	Mean distance	Rainy season	Autumn season	Mean distance	Rainy season	Autumn season	Mean distance
D1	83.13±0.11	81.15±0.1	82.14	16.89±0.02	18.85±0.01	17.85	18.67±0.5	16.67±0.5	17.67
D2	84.65±0.11	81.40±0.005	83.07	15.35±0.01	18.51±0.05	16.93	19.67±0.5	19.33±1.1	19.5
D3	85.18±0.9	84.09±0.09	84.64	14.81±0.9	15.90±0.09	15.36	20±0.5	20±1.7	20.
DC	91.04±0.6	87.30±1.4	89.17	12.70±1.4	8.95±0.6	10.82	20.33±1.5	23±1	21.67
Mean season	86.00	83.40		14.94	15.55		19.667	19.75	
C.D. at 5%			C.D. at 5%			C.D. at 5%			

Between distance (D)	0.856	s	Between distance (D)	0.855	S	Between distance (D)	1.378	S
Between season (S)	0.605	S	Between season (S)	0.605	S	Between season (S)	N/A	NS
Interactions (D x S)	1.211	S	Interactions (D x S)	1.210	s	Interactions (D x S)	1.948	S

S= Significant at 5% probability level; NS = Non Significant

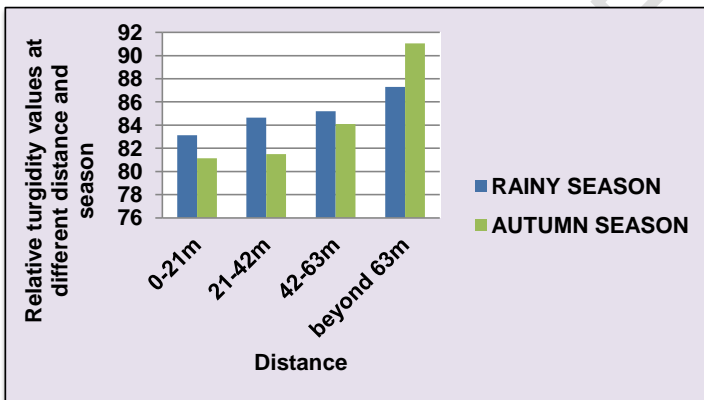


Fig. 1.1:- Relative turgidity (%) at different distances and season

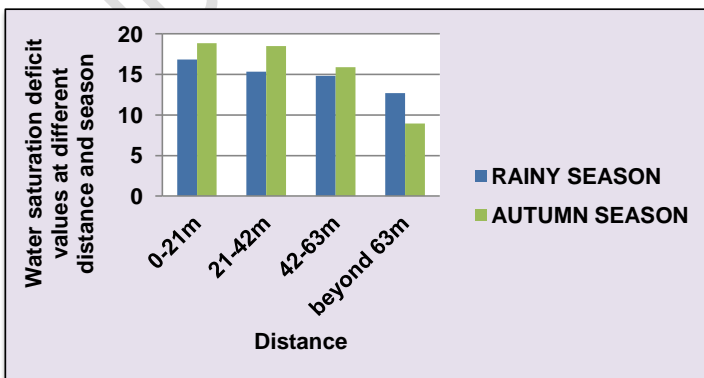


Fig. 1.2:- Water saturation deficit (%) at different distances and season

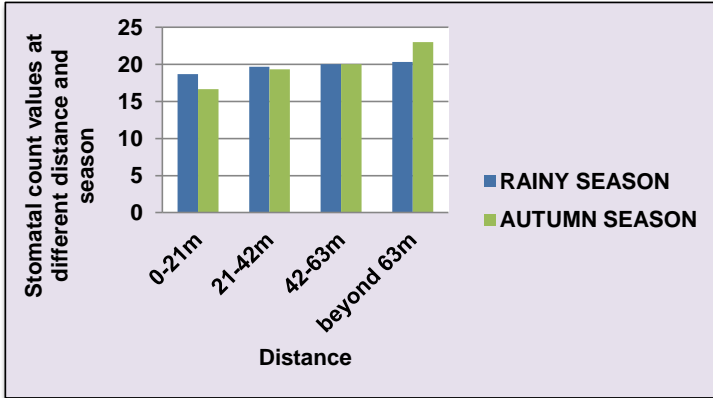


Fig. 1.3:- Stomatal count (numbers) at different distances and season

Table 4: Plant physiological parameters (ii)

Season distance	Leaf area (cm ²)			Specific leaf weight (mg cm ²)		
	Rainy season	Autumn season	Mean distance	Rainy season	Autumn season	Mean distance
D1	32.28±0.2	30.84±0.05	31.56	7.7±0.2	6.9±0.1	7.3
D2	33.03±0.1	31.71±0.1	32.37	7.8±0.1	7.3±0.2	7.5
D3	33.2±0.5	32.55±0.1	32.87	7.6±0.1	7.4±0.3	7.5
DC	34.02±0.1	33.71±0.03	33.86	7.7±0.1	7.4±0.2	7.5
Mean season	33.13	32.2		7.7	7.2	
C.D. at 5%				C.D. at 5%		
Between distance (D)	0.274	S		Between distance (D)	N/A	NS

Between season (S)	0.193	S	Between season (S)	0.184	S
Interactions (D x S)	0.387	S	Interactions (D x S)	N/A	NS

S= Significant at 5% probability level; NS = Non Significant

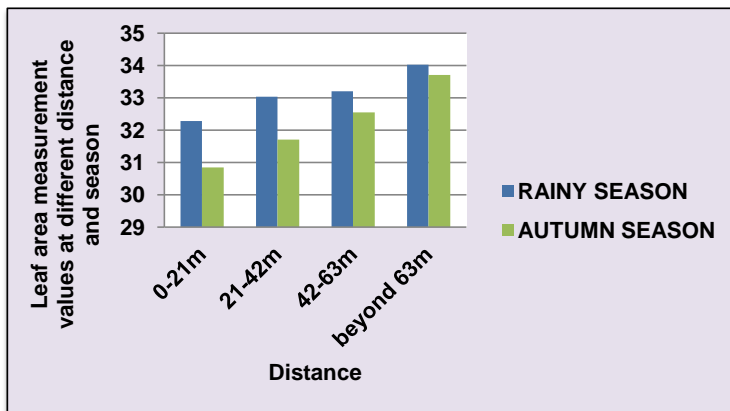


Fig. 2.1 :- Leaf area measurement (cm²) at different distances and season

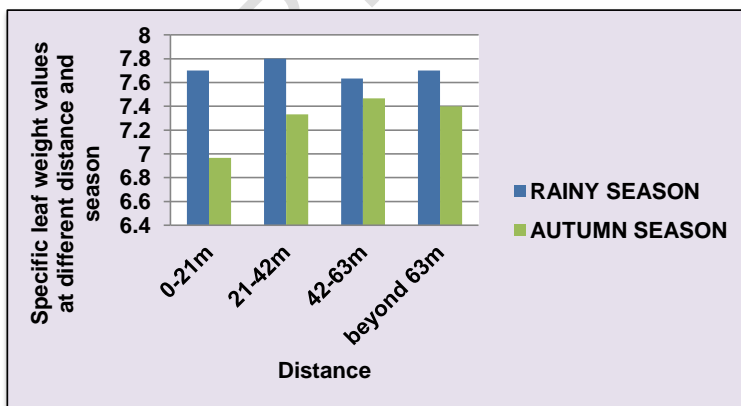


Fig. 2.2:- Specific leaf weight (mg cm⁻²) at different distances and season

3.2 Soil parameters

3.2.1 Soil physical parameters

3.2.1.1 Bulk density

From the present experiment, it was observed that the bulk density of the experimental plot varied significantly with respect to distance from the oil effluent point. Among the distances, D1 showed maximum bulk density content both in the rainy season and autumn season i.e. 1.33 Mg m⁻³ and 1.31 Mg m⁻³ (table 5, figure 3.1). The lowest Bulk density was recorded in Dc in both the rainy and autumn seasons (1.22 Mg m⁻³ and 1.22 Mg m⁻³ respectively) (table 5, figure 3.2). When seasons are considered, then no significant difference was observed in bulk density content. Among the distances, D3 and Dc were highly significant.

Results were in agreement with [21] They reported that the bulk density of the crude oil-contaminated soil showed higher values than the control site showing that the viscous crude oil settled into the pores to increase both the soil's wet weight and the liquid content. [33] reported that the presence of hydrocarbons resulted in compactness of the soil particles and an increase in the bulk density of soils.

3.2.1.2 Porosity

Soil porosity values of the experimental plot varied with respect to distance from the oil effluent point and were found to be lower near the spilled point whereas increased gradually with an increase in distance. However, among the distances, Dc and D3 were highly significant. Among the distances, Dc showed maximum soil porosity content both in the rainy season and autumn season i.e. 49.7% (table 5, figure 3.2). The lowest soil porosity content was recorded in D1 in both rainy and autumn seasons, i.e. 44.9% (table 5, figure 3.2) There was no seasonal variation observed in soil porosity

The lower values of porosity in the crude oil spilled areas could possibly be due to the formation of a thick crude oil coating above the soil surface which might have resulted in compactness of soil particles and thus reduces the porosity of oil spilled areas. Also, high bulk density is an indicator of low soil porosity and soil compaction [34]). [33] explained that crude oil pollution hampers soil physical properties and pore spaces might be clogged which reduced soil porosity resulting in poor aeration, slow infiltration of water into the soil, increased bulk density ultimately affecting plant growth.

3.2.1.3 Hydraulic conductivity

From the present experiment, it was observed that the soil hydraulic conductivity content varies significantly concerning distance from the crude oil effluent point. Among the distances, Dc and D3 were highly significant When seasons are considered, then no significant difference was observed in soil hydraulic conductivity content Among the distances Dc showed maximum soil hydraulic conductivity content both in the rainy season and autumn season i.e. 0.35 cm min⁻¹ and 0.33 cm min⁻¹(table 5, figure 3.3). The lowest soil hydraulic conductivity was recorded in D1 in both rainy and autumn seasons (0.29 cm min⁻¹ and 0.3 cm min⁻¹ respectively) (table 5, figure 3.3)

The increase in hydraulic conductivity with increasing distance from oil effluent contaminated area was possibly due to the corresponding decrease in crude oil contamination; Wetting of and entry of water into the soil was reduced with an increase in oil contamination which could be due to the hydrophobic coating of oil around the soil particles, consequently causing a reduction in soil hydraulic conductivity. The results were in agreement with [21] They reported that the polluted soil sample had comparatively less hydraulic conductivity

values than the control soil sample due to blockage of polluted soils pores by oil films. [35] in their experiment explained that the oil coating around the soil particles, it reduces the availability of water to the plant roots because of the gradient development between the soil particles and pore spaces. Pore space may be occupied and pore connectivity trapped by oil content instead of water molecules. As a result, the moisture flow from soil to root is reduced, and sometimes a reverse flow occurs, due to which plant growth is adversely affected.

Table 5: soil physical parameters

Season distance	Soil Bulk density(mg m-3)			Soil Porosity(%)			Hydraulic conductivity(cm min-1)			
	Rainy season	Autumn season	Mean distance	Rainy season	Autumn season	Mean distance	Rainy season	Autumn season	Mean distance	
D1	1.33 ±0.02	1.31±0.01	1.32	44.28 ±1.9	45.1±1.01	44.99	1.64±0.07	1.28±0.02	1.46	
D2	1.32 ±0.005	1.3±0.01	1.31	44.83 ±1	45.86 ±1.1	45.46	1.58±0.03	1.48±0.01	1.53	
D3	1.27 ±0.01	1.28±	1.27	47.07 ±0.9	46.46 ±0.8	46.76	1.78±0.1	1.71±0.02	1.75	
DC	1.22 ±0.02	1.22±0.01	1.22	50.4±0.1	49.17 ±0.01	49.78	2.1±0.02	2.02±0.1	2.06	
Mean season	1.288	1.282		46.64	46.65		1.781	1.62		
C.D. at 5%				C.D. at 5%				C.D. at 5%		
Between distance (D)	0.019	S		Between distance (D)	0.926	S		Between distance (D)	0.014	S
Between season (S)	N/A	NS		Between season (S)	N/A	NS		Between season (S)	N/A	NS

Interactions (D x S)	N/A	NS	Interactions (D x S)	N/A	NS	Interactions (D x S)	N/A	NS
----------------------	-----	----	----------------------	-----	----	----------------------	-----	----

S= Significant at 5% probability level; NS = Non Significant

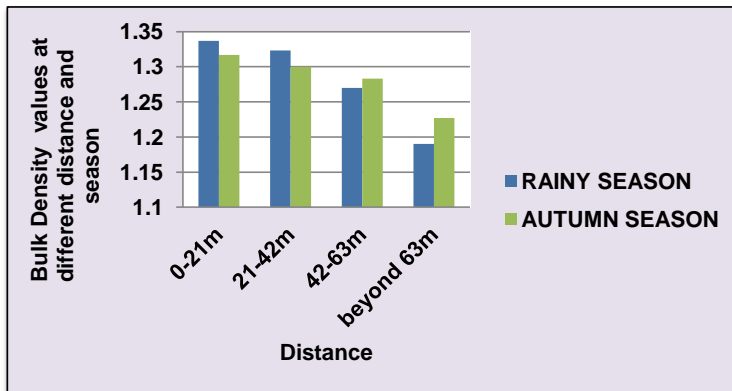


Fig. 3.1:- Bulk Density (mg m^{-3}) at different distances and season

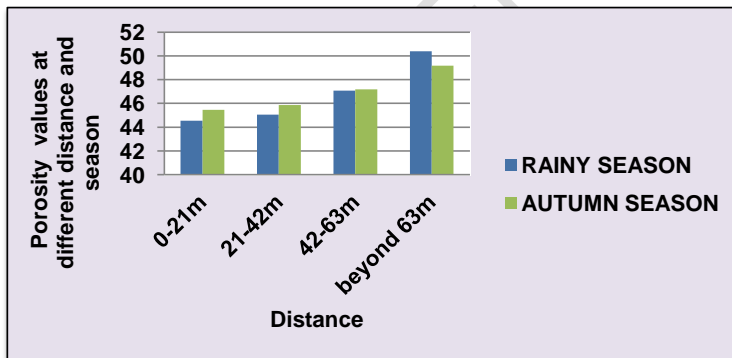


Fig. 3.2:- Porosity (%) at different distances and season

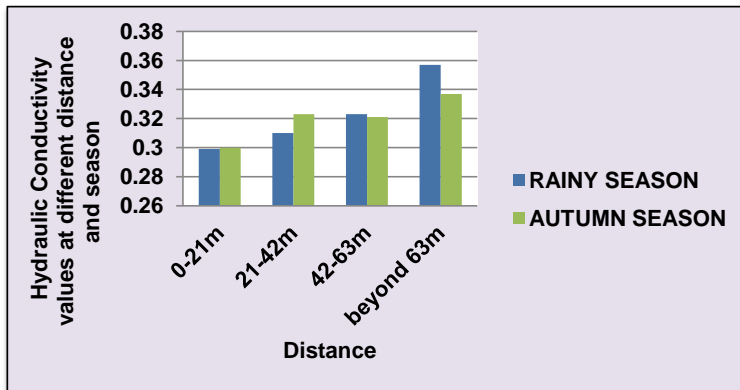


Fig. 3.3:- Hydraulic Conductivity (cm m^{-1}) at different distances and season

3.2.1.4 Soil particle analysis

The texture of the soil of the experimental plot, i.e. Shalmari-1, Dibrugarh is presented in table 6. According to the result, all samples fall within the sandy clay loam textural class. The sand content of the soil ranged from 74.2% to 74.7%, silt from 2.6% to 3.2%, and clay content ranged from 22.1% to 22.9% (table 6, figure 4.1) The soil particle analysis had not shown any variation with respect to both distance and season. Thus, it was observed that the presence or absence of crude oil didn't affect the soil texture. The findings were supported by [36], who reported no significant difference between the soil texture of polluted and unpolluted since soil texture is an inherent property of soil, and cannot be altered by the presence of pollutants

Table 6: soil particle analysis

Season distance	Sand			Silt			Clay		
	Rain y seas on	Autum n seas on	Mean distan ce	Rainy seaso n	Autum n seas on	Mean distan ce	Rainy seaso n	Autum n seas on	Mean distan ce
D1	74.23	74.28	74.255	3.223	3.253	3.238	22.55	22.47	22.51
D2	74.307	74.29	74.298	3.25	3.277	3.263	22.443	22.433	22.438
D3	74.32	74.313	74.317	3.33	3.29	3.31	22.38	22.417	22.398
DC	74.6	74.7	74.65	2.6	3.2	2.9	22.9	22.1	22.5

Mean season	74.364	74.396		3.101	3.255		22.568	22.355		
C.D. at 5%				C.D. at 5%				C.D. at 5%		
Between distance (D)	N/A	NS		Between distance (D)	N/A	NS	Between distance (D)	N/A	NS	
Between season (S)	N/A	NS		Between season (S)	N/A	NS	Between season (S)	N/A	NS	
Interactions (D x S)	N/A	NS		Interactions (D x S)	N/A	NS	Interactions (D x S)	N/A	NS	

S= Significant at 5% probability level; NS = Non Significant

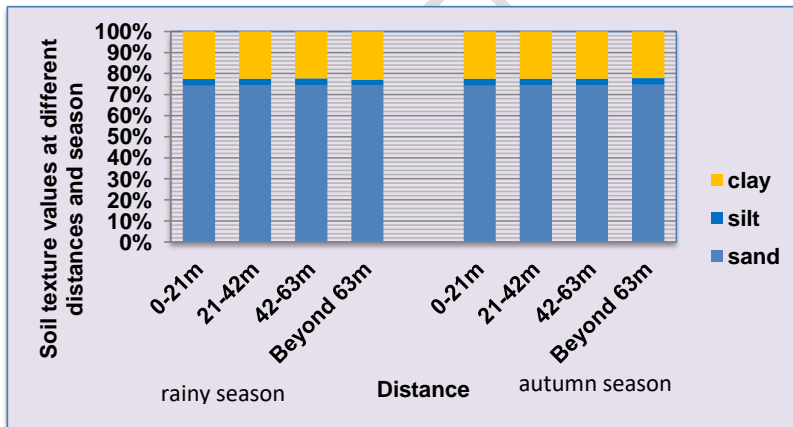


Fig. 4.1:- Soil particle analysis (%) at different distances and season

3.2.2 Soil chemical parameters

3.2.2.1 pH

The pH values of the soil of the experimental plot are presented in table 7, figure 5.1. It was observed that the soil was medium acidic in nature. From the present experiment, it was

observed that the soil pH content varies significantly in different distances from the crude oil effluent point. When seasons were considered, then no significant difference was observed in soil pH content. It was more or less the same in all the distances. However, the maximum pH value was recorded in the distance D1 i.e. 5.92, and the minimum in the distance DC, i.e. 5.76. Among the distances, D3 and Dc was highly significant

The higher pH of oil-polluted soils was possibly due to the presence of hydrocarbon; it hinders the leaching of basic salts by binding which then probably posed a major resistance to the removal of such basic ions [37;38].

The findings were in agreement with [21] who worked on *Vigna unguiculata* and reported that contamination of soil with crude oil showed a 26% increase in the soil pH value because of the bacterial decomposition of oil in the anaerobic environment due to oil-blocked soil pores. This change signified that spilling crude oil on agricultural soil tended to buffer the polluted soil towards neutral pH [39]. [40] observed a gradual increase in soil pH with increased oil concentrations. The effect was attributed to the accumulation of exchangeable bases in the oil-polluted soils. This affected the ionic stability of the soil and conversely nutrient availability and uptake by crop plants

3.2.2.2 Organic carbon content

The results revealed that the soil organic carbon content of soils was maximum at the effluent point, i.e. D1 (13.03 g kg⁻¹) and it decreased gradually with the increase in distance from the drilling point. The lowest soil organic carbon content was at DC, i.e. 11.2 g kg⁻¹. From the present experiment, it was observed that the soil organic carbon content varies significantly concerning distance from the crude oil effluent point. Among the distances, Dc and D2 were highly significant. When seasons were considered, then no significant difference was observed in soil organic carbon content, presented in table 7, figure 5.2

The possible reason for the increase in organic carbon content in polluted points could be due to an increase in the crude oil contamination. This was due to the presence of petroleum hydrocarbons in the crude oil, which might have resulted in the agronomical addition of the carbon content to the soil [35]. The findings were in agreement with [21]. They reported that contamination of soil with crude oil showed an increase in the organic carbon in the contaminated soil than the control soil because of the bacterial decomposition of oil in the anaerobic environment due to oil-blocked soil pores. [4] reported that the increase of organic carbon is directly proportional to the increase of crude oil added to the soil due to the release of carbon from spilled crude oil

3.2.2.3 Electrical conductivity

From the present experiment, it was observed that the soil electrical conductivity content varies significantly for distance from the crude oil effluent point. Among the distances, D1 and D3 were highly significant when seasons are considered, and then no significant difference was observed in soil electrical conductivity content. According to the results, electrical conductivity was found highest in the distance D1 for both rainy and autumn season, i.e. 0.33 dS m⁻¹ and 0.34 dS m⁻¹ respectively and lowest electrical conductivity was found in unpolluted site DC for both rainy and autumn season, i.e. 0.28 and 0.29 respectively (table 7, figure 5.3).

The possible reason for the increase in electrical conductivity content in near distance to the polluted point could be due to the accumulation of exchangeable bases in the oil-polluted soils. It affects the ionic stability of the soil and conversely nutrient availability and uptake by crop plants [40]. These findings were supported by [35]. They reported that as crude oil consists of petroleum hydrocarbons, a considerable amount of ions could bond with the existing ions in the soil.

Table 7: Soil chemical parameters (i)

Season distance	Soil pH			Soil organic carbon content (g kg ⁻¹)			Electrical conductivity (ds m ⁻¹)			
	Rainy season	Autumn season	Mean distance	Rainy season	Autumn season	Mean distance	Rainy season	Autumn season	Mean distance	
D1	5.94 ±0.01	5.91±0.01	5.925	13.06 ±0.03	13±0.02	13.03	0.33±0.01	0.34±0.001	0.33	
D2	5.85 ±0.03	5.83±0.005	5.842	12.03 ±0.05	11.8±0.01	11.917	0.32±0.002	0.32±0.02	0.32	
D3	5.82 ±0.1	5.78±0.02	5.803	12.7±0.1	12.63 ±0.2	12.667	0.3±0.004	0.31±0.001	0.3	
DC	5.76 ±0.01	5.76±0.02	5.763	11.23 ±0.1	11.3±0.2	11.267	0.28±0.01	0.29±0.01	0.28	
Mean season	5.84	5.82		12.24	12.19		0.31	0.31		
C.D. at 5%				C.D. at 5%				C.D. at 5%		
Between distance (D)	0.056	S		Betwe en distance (D)	0.165	S	Betwe en distance (D)	0.013	S	
Between season (S)	N/A	NS		Betwe en season (S)	N/A	NS	Betwe en season (S)	N/A	NS	

Interactions (D x S)	N/A	NS	Interactions (D x S)	N/A	NS	Interactions (D x S)	N/A	NS
----------------------	-----	----	----------------------	-----	----	----------------------	-----	----

S= Significant at 5% probability level; NS = Non Significant

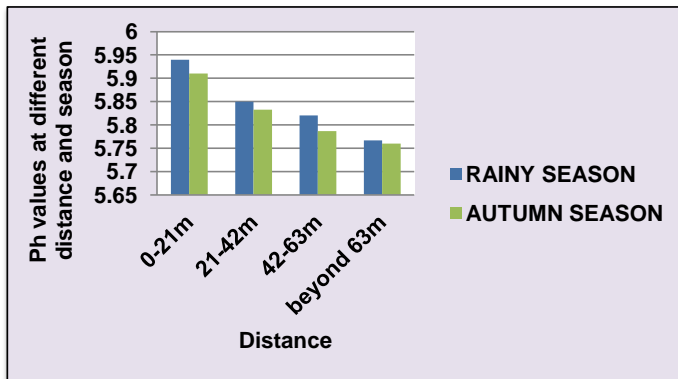


Fig. 5.1:- Ph at different distances and season

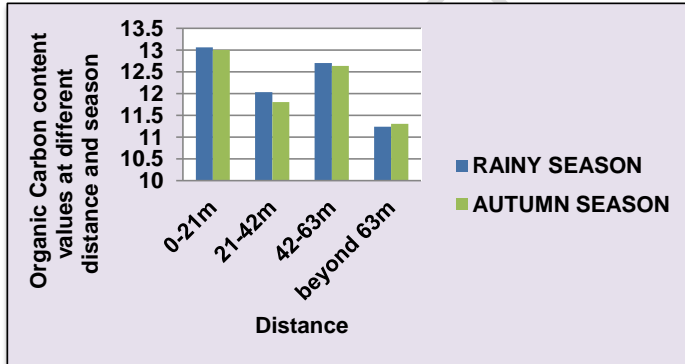


Fig. 5.2:- Organic Carbon content (g kg⁻¹) at different distances and season

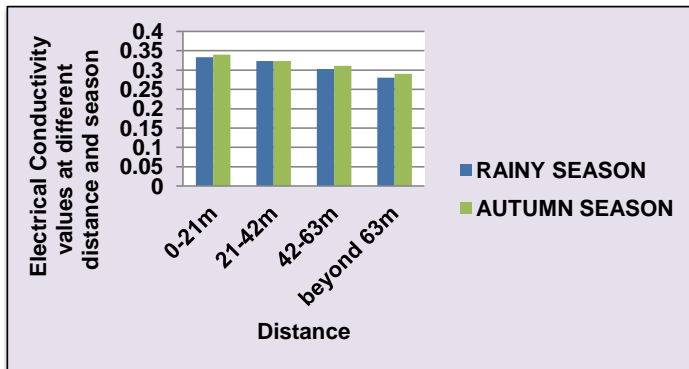


Fig. 5.3:- Electrical Conductivity (dS m⁻¹) at different distances and season

Soil available nutrients

3.2.2.4 Available nitrogen

From the present experiment, it was observed that amount of available nitrogen content of soil varied significantly in different distances from the crude oil effluent point. When seasons are considered, then no significant difference was observed in available nitrogen content. Maximum available nitrogen content was recorded in the distance D2 i.e. 775.6 kg ha⁻¹ and minimum in the distance DC i.e. 681.1 kg ha⁻¹ (table 8, figure 6.1). Among the distances, D3 and DC were highly significant. The findings were in agreement with [21] who reported that contamination of crude oil increased the available nitrogen content of the soil by 33% to 103% over the control soil. This was due to bacterial decomposition of oil in the anaerobic environment due to oil-blocked soil pores.

3.2.2.5 Available phosphorous

From the present experiment, it was observed that amount of available phosphorous content of soil varied significantly in different distances from the crude oil effluent point. Among the distances, D1 and D3 were highly significant. When seasons are considered, then no significant difference was observed in available phosphorous content. Maximum available phosphorous content was recorded in the distance D1 i.e. 40.9 kg ha⁻¹ and minimum in the distance DC i.e. 35.6 kg ha⁻¹ (table 8, figure 6.2). The findings were in agreement with [21] They reported that contamination of soil with crude oil showed a 33% to 103% increase in the available phosphorous content than the control soil because of the bacterial decomposition of oil in the anaerobic environment due to oil-blocked soil pores. The observed increase in available phosphorus also confirmed earlier reports by [41]

3.2.2.6 Available potassium

Soil available potassium increased with increased oil effluent concentration and decreased with distance. Available potassium was maximum near the drilling point D1 i.e. 40.9 kg ha⁻¹ whereas minimum available potassium content was found in the distance DC i.e. 35.6 kg ha⁻¹ (table 8, figure 6.3). It was observed that amount of available phosphorous content of soil varied significantly in different distances from the crude oil effluent point. Among the distances, D1 and D3 were highly significant. When seasons were considered, then no

significant difference was observed in available phosphorous content. The possible reason for the increase in potassium content in the oil spilled soil may be due to the fact that sodium and potassium salts are being used in drilling operations, which find their way to the nearby water bodies along with the effluents and thus an ionic concentration may build-up resulting in more potassium in crude oil-contaminated soil. Further, deposition of more potassium in deeper soils takes place which moved in the top layer of the soil during the oil drilling process and got deposited in the top layer of the soil near the effluent point [42]

Table 8: soil chemical parameters (ii)

Season distance	Available nitrogen content (kg ha ⁻¹)			Available phosphorous content (kg ha ⁻¹)			Available potassium content (kg ha ⁻¹)			
	Rainy season	Autumn season	Mean distance	Rainy season	Autumn season	Mean distance	Rainy season	Autumn season	Mean distance	
D1	773.7±0.3	774.4±0.1	774.1	40.8±0.2	41.1±0.2	40.923	230.7±0.5	233.6±0.7	232.1	
D2	776.7±0.5	774.4±1.4	775.6	40.6±0.6	39.8±3.4	40.228	228.2±	223.5±0.6	225.9	
D3	769.9±4	771.2±0.1	770.5	37.7±0.1	37.9±0.0	37.872	220±9.1	210.4±9.1	215.2	
DC	681.1±0.01	681.2±0.01	681.1	35.6±0.01	35.7±0.01	35.695	214.5±0.01	215.1±0.01	214.8	
Mean season	750.3	750.3		38.79	38.63		223.3	220.6		
C.D. at 5%				C.D. at 5%				C.D. at 5%		
Between distance (D)	1.966	S		Between distance (D)	1.559	S	Between distance (D)	6.678	S	
Between season (S)	N/A	NS		Between season (S)	N/A	NS	Between season (S)	N/A	NS	

Interactions (D x S)	N/A	NS	Interactions (D x S)	N/A	NS	Interactions (D x S)	N/A	NS
----------------------	-----	----	----------------------	-----	----	----------------------	-----	----

S= Significant at 5% probability level; NS = Non Significant

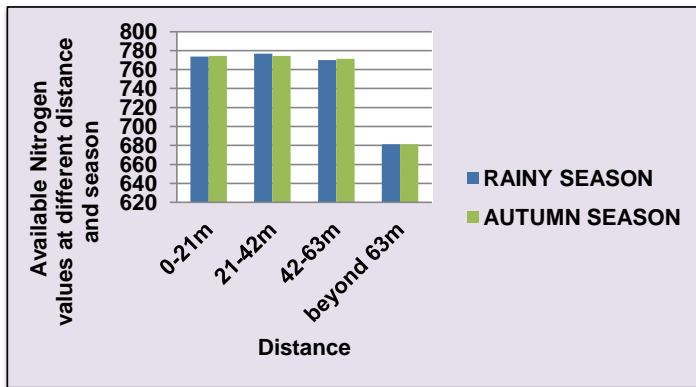


Fig. 6.1:- Available Nitrogen (kg ha⁻¹) at different distances and season

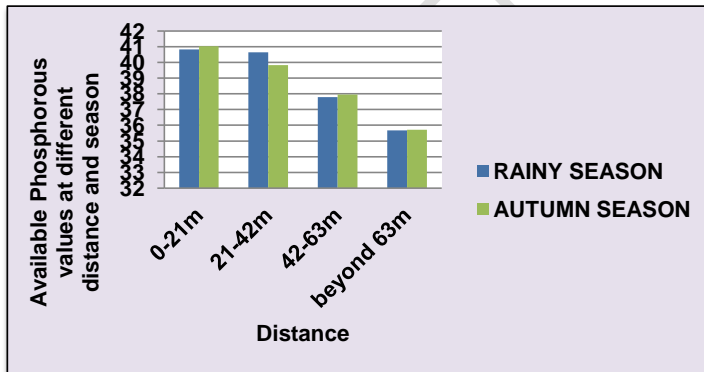


Fig. 6.2:- Available Phosphorous (kg ha⁻¹) at different distances and season

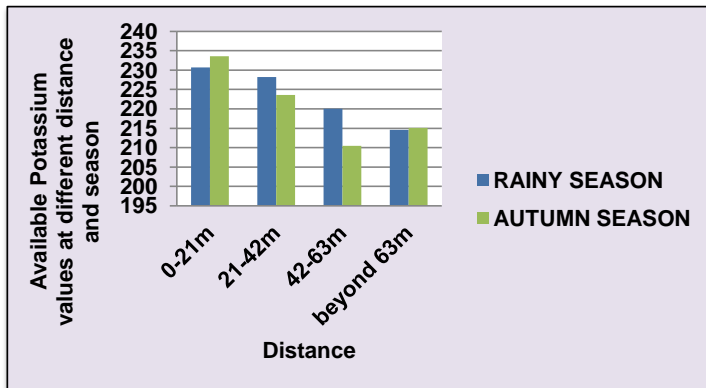


Fig. 6.3:- Available Potassium (kg ha⁻¹) at different distances and season

4. CONCLUSION

The results obtained from the present investigation revealed that oil effluent was observed to be a major factor for damaging plant health as well as soil quality in Shalmari-1 gaon and the impact was more prominent near the effluent point. Due to the presence of oil effluent, the oil particles block the soil pores, which led to a water stress condition. A gradual decline in relative turgidity, stomata count, leaf area, plucking point density, number of primaries, total chlorophyll, caffeine, and total polyphenol content was observed. In the case of soil parameters, there was a decrease in porosity, hydraulic conductivity content, whereas the increase in bulk density, organic carbon content, electrical conductivity. Overall, it can be concluded that oil contaminated soils are harmful to the growth of tea as well as soil health, hence any remedial measures such as phytoremediation [3,4], bioremediation [43], etc. should be followed in such soils.

The present investigation was studied to know the effects of oil effluent on plant and soil health. The study resulted in negative effects on the growth of tea plants and soil health.

One of the most prospective techniques for the restoration of oil-contaminated soils is phytoremediation. It is environmentally friendly and cost-effective. Phyto-remediation by planting *Calamagrostis angustifolia*, *Cyperus brevifolius*, etc. has the potential to simultaneously restore and remediate the petroleum hydrocarbon-contaminated wetlands [3;4] Also, Bioremediation of contaminated soil using poultry manure sawdust, yeast extract will be effective to decontaminate the polluted soil. [43]

Competing Interest

No conflict of interest or conflict of interest exists among the authors

Formatted: Font: (Default) Times New Roman, 12 pt

Comment [DK5]: Mention whether conflict of interest exist or not among the authors.

Formatted: Highlight

Formatted: Highlight

REFERENCES

1. Udo, E. J., & Fayemi, A. A. A. The Effect of Oil Pollution of Soil on Germination, Growth and Nutrient Uptake of Corn 1. *Journal of Environmental Quality*. 1975; 4(4): 537-540.
2. Schutzenhubel A, Polle A. Plant responses to abiotic stresses: heavy metal-induced oxidative stress and protection by mycorrhization *J. Expt. Bot.* 2002;53(372): 1351-1365
3. Wang, Y., Feng, J., Lin, Q., Lyu, X., Wang, X., & Wang, G. Effects of crude oil contamination on soil physical and chemical properties in Momoge wetland of China. *Chinese Geographical Science*. (2013);23(6):708-715.
4. Barua, D., Buragohain, J., & Sarma, S. K. Certain Physico-chemical changes in the soil brought about by contamination of crude oil in two oil fields of Assam, NE India. *European Journal of Experimental Biology*. (2011);1(3): 154-161.
5. Weatherley, P.. Studies in the water relations of the cotton plant. I. The field measurement of water deficits in leaves. *New Phytologist*. (1950); 81-97.
6. Barrs, H. D., & Weatherley, P. E. A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Australian journal of biological sciences*. (1962); 15(3): 413-428.
7. Čatský, J. Determination of water deficit in disks cut out from leaf blades. *Biologia Plantarum*. (1960); 2(1): 76.
8. A B. Beakbane and P. K. Majumdar.. A relationship between stomatal density and growth potential in apple rootstocks. *J. Hort. Sei.* 1975; 59: 285-289.
9. Pandey, S. K., & Singh, H. A simple, cost-effective method for leaf area estimation. *Journal of botany*, 2011.
10. Robinson, D., & Dobrenz. *Specific Leaf Weights: Daily Trends in an Alfalfa Field*. 1986
11. Blake, G. R., & Hartge, K. H.. Bulk density. *Methods of soil analysis: Part 1 Physical and mineralogical methods*. 1986;5:363-375
12. Barua, T.C and Barthakur, H.P. A text book of soil analysis. Vikash publishing, PVT Ltd, New Delhi. 1997.
13. Jackson, M.L.. *Soil Chemical Analysis*. Prentice Hall of India Pvt. Ltd., New Delhi-1. 1973
14. Allison, LE in Black, CA et al. 1965, *Methods of Soil Analysis*, pp1372-1378
15. Piper, CS, *Soil and Plant Analyses*. University of Adelaide. 1942
16. Subbiah, B. V., & Asija, G. L. A rapid method for the estimation of nitrogen in soil. *Current Science*. 1956; 26: 259-260.

Comment [DK6]: Add 1 or 2 references from International Journal of Plant & Soil Science, if you found suitable.

Comment [DK7]: Avoid/ remove older than 2000. Keep these references only in very necessary case.

Comment [DK8]: Write the abbreviated form of Journal Name.

Comment [DK9]: Remove bracket

Comment [DK10]: Remove bracket. Correct/revise all the references accordingly. Strictly follow the Journal Style

17. Han, G., Cui, B. X., Zhang, X. X., & Li, K. .. The effects of petroleum-contaminated soil on photosynthesis of *Amorpha fruticosa* seedlings. *International journal of environmental science and technology*. (2016);13(10): 2383-2392.
18. Merkl, N., Schultze-Kraft, R., & Infante, C. Phytoremediation in the tropics—the effect of crude oil on the growth of tropical plants. *Bioremediation Journal*. 2004; 8 (3-4): 177-184.
19. Merkl, N., Schultze-Kraft, R., & Infante, C. Assessment of tropical grasses and legumes for phytoremediation of petroleum-contaminated soils. *Water, air, and soil pollution*. 2005; 165(1-4): 195-209.
20. Upadhyaya, H., & Panda, S. K. Abiotic stress responses in tea [*Camellia sinensis* L (O) Kuntze]: an overview. *Reviews in Agricultural Science*. 2013; 1: 1-10.
21. Essien, O. E., & John, I. A. Impact of crude-oil spillage pollution and chemical remediation on agricultural soil properties and crop growth. *Journal of Applied Sciences and Environmental Management*. 2010; 14(4).
22. Naidoo, G., Naidoo, Y., & Achar, P. Responses of the mangroves *Avicennia marina* and *Bruguiera gymnorrhiza* to oil contamination. *Flora-Morphology, Distribution, Functional Ecology of Plants*. 2010; 205(5): 357-362.
23. Reis, D. J. C. *Environmental control in petroleum engineering*. Elsevier. 1996.
24. Suprayogi, B., & Murray, F. A field experiment of the physical and chemical effects of two oils on mangroves. *Environmental and experimental Botany*. (1999);42(3): 221-229
25. Baker, J. M. The effects of oils on plants. *Environmental Pollution* 1970; 1(1): 27-44.
26. Pezeshki, S. R., Hester, M. W., Lin, Q., & Nyman, J. A. The effects of oil spill and clean-up on dominant US Gulf coast marsh macrophytes: a review. *Environmental pollution*. 2000; 108(2): 129-139.
27. Gill, L. S., Nyawuame, H. G. K., & Ehihametalor, A. O. Effect of crude oil on the growth and anatomical features of *Chromolaena odorata* (L.) K. & R. *Newsletter*. 1992;6(1): 1-6.
28. Baruah, P., Saikia, R. R., Baruah, P. P., & Deka, S. Effect of crude oil contamination on the chlorophyll content and morpho-anatomy of *Cyperus brevifolius* (Rottb.) Hassk. *Environmental Science and Pollution Research*. (2014); 21(21): 12530-12538.
29. Cochard, H., Coll, L., Le Roux, X., & Améglio, T. Unraveling the effects of plant hydraulics on stomatal closure during water stress in walnut. *Plant physiology*. 2002; 128(1): 282-290.
30. Okonokhua, B. O., Ikhajagbe, B., Anoliefo, G. O., & Emede, T. O. The effects of spent engine oil on soil properties and growth of maize (*Zea mays* L.). *Journal of Applied Sciences and Environmental Management*. 2007; 11(3).
31. Lopes, A., da Rosa-Osman, S. M., & Piedade, M. T. F. Effects of crude oil on survival, morphology, and anatomy of two aquatic macrophytes from the Amazon floodplains. *Hydrobiologia*. 2009;636(1): 295-305.

32. Larcher, W. *Physiological plant ecology: ecophysiology and stress physiology of functional groups*. Springer Science & Business Media. 2003.
33. Abosede, E. E. Effect of crude oil pollution on some soil physical properties. *Journal of Agriculture and Veterinary Science*. 2013; 6(3): 14-17.
34. Borah, K. K., Bhabajit, B., & Sarma, H. P. Variation of bulk density and organic matter in soils of tea garden belt of undivided Darrang district, Assam. *Archives of applied science research*. 2009; 1(2): 159-164
35. Devatha, C. P., Vishal, A. V., & Rao, J. P. C. Investigation of physical and chemical characteristics on soil due to crude oil contamination and its remediation. *Applied Water Science*. 2019; 9(4): 89.
36. Ihem, E. E., Osuji, G. E., Onweremadu, E. U., Uzoho, B. U., Nkwopara, U. N., Ahukemere, C. M., ... & Okoli, N. H. Variability in selected Properties of Crude Oil-Polluted Soils of Izombe, Northern Niger Delta, Nigeria. *Agriculture, Forestry and Fisheries*. 2015;4(3): 29.
37. Ogboghodo, I. A., Erebor, E. B., Osemwota, I. O., & Isitekhale, H. H. The effects of application of poultry manure to crude oil polluted soils on maize (*Zea mays*) growth and soil properties. *Environmental Monitoring and assessment*. 2004; 96(1-3): 153-161.
38. Okoro, D., Oviasogie, P. O., & Oviasogie, F. E.. Soil quality assessment 33 months after crude oil spillage and clean-up. *Chemical Speciation & Bioavailability*. 2011;23(1): 1-6.
39. Ellis Jr, R., & Adams Jr, R. S. Contamination of soils by petroleum hydrocarbons. In *Advances in agronomy*, Academic Press.. 1961;13;197-216
40. Uquetan, U. I., Osang, J. E., Egor, A. O., Essoka, P. A., Alozie, S. I., & Bawan, A. M. A case study of the effects of oil pollution on soil properties and growth of tree crops in Cross River State, Nigeria. *Int Res J Pure Appl Phys*. 2017; 5(2): 19-28
41. Toogood, J. A., & University of Alberta. Dept. of Soil Science.. *The reclamation of agricultural soils after oil spills*. Department of Soil Science, University of Alberta. 1977
42. Deka, S., & Devi, A. Impact of flaring of group gathering station (GGS) on physico-chemical properties of rice field soil at Lakowa oil field of Assam. *Poll. Res*. 1994; 13(2): 101-105.
43. Amadi, A., Abbey, S. D., & Nma, A. Chronic effects of oil spill on soil properties and microflora of a rainforest ecosystem in Nigeria. *Water, Air, and Soil Pollution*, 1996. 86(1-4), 1-11.