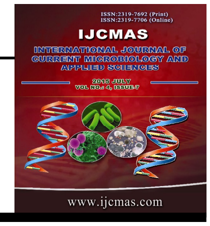


International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 7 (2015) pp. 848-861**
http://www.ijcmas.com

Original Research Article

Augmentation of biodegradability of bacterial isolates by utilizing agricultural fertilizers and their comparative analysis

Umema Mohsin[2]*, Madan Lowry[1], Preety Shrivastava[2] and Ankit Chokriwal[1]

[1]University of Rajasthan (Centre for Advanced Studies), Jaipur, India
[2]Mahatam Joyti Rao Phule University, Jaipur, India
**Corresponding author*

Keywords

Bioremediation, Fertilizer, NPK, Mineralization, hydrocarbon

A B S T R A C T

The main aim of this study was to identify whether agricultural fertilizers (NPK) enhances the microbial degradation of hydrocarbons in soil. The present study reveal that effect of inorganic compound Na_2CO_3 and agriculture fertilizers NPK in bioremediation of petroleum contaminated soil samples of Jaipur. We used three contaminated soil sample labeled as T1, T2 and T3. The Na_2CO_3 concentration 10mg, 20mg and 40 mg, NPK Concentration 10g, 20g and 40g were added for bioremediation. The maximum concentration of Na_2CO_3 shows CO_2 evolved for soil sample T1, T2 and T3 is 30.96mg, 50.6mg, 90.64mg and carbon mineralization is 8.44mg, 13.8mg and 24.65mg respectively. In case of NPK CO_2 evolved is 46.50g, 60.2g and 78.5g and carbon mineralization 12.68g, 16.4g and 21.4g respectively. Increase in CO_2 evolution and carbon mineralization shows that increase in the microbial population and enhancement of metabolic activity to utilization of hydrocarbons. The chloride and bicarbonate concentration also increased with the concentration of Na_2CO_3 and NPK.

Introduction

Soil is considered as the largest terrestrial carbon pool (Post *et al*., 1982). Stored soil carbon pool is an outcome of an imbalance between organic matter produced by plants and its decomposition back into the atmosphere as carbon dioxide (CO_2). Hence with this concept, Soil respiration is defined as a measure of released CO_2 from the soil as a result of decomposition of soil organic matter (SOM) by soil microbes and respiration from plant roots and soil fauna. It indicates the level of microbial activity, SOM content and its decomposition and acts as an important indicator of soil health, nutrients contained in organic matter being converted to inorganic forms that are available for plant uptake. This conversion is known as mineralization. Soil respiration is also referred as carbon mineralization (Esteban *et al*., 2000; J. Curiel *et al*., 2007)

Soil respiration reflects the capacity of soil to sustain soil life such as crops, soil animals, and microorganisms (Snežana Maletić *et al*., 2013). After tillage excessive respiration and SOM decomposition occurs due to destruction of soil aggregates that previously protected SOM and increased soil aeration. Depleted SOM, reduced soil aggregation, and limited nutrient availability for plants and microorganisms can cause reduced crop production. Inherent factors that impact soil respiration, such as climate which can not be changed. But now a days it is vulnerable to global warming. Inherent soil respiration rates depend on amount and quality of SOM, temperature, salinity, pH, moisture and aeration (J. Curiel *et al*., 2007).

Diesel pollution generated from humanity is a widespread environmental problem. It contains various components including polar hydrocarbons, n-alkanes, unresolved complexes of branched- and cyclo-alkanes as well as aromatics (Killops and Al-Jaboori,1990). The environmental risk is associated with mutagenic properties and their resistance toward degradation (WHO, 1983) which affects soil use and fertility and leads to unavailability of nutrients and elements (Anoliefo *et al*., 2003) and dehydration. Phytoremediation involves the use of plants to stabilize hydrocarbon polluted soils and to enhance hydrocarbon degradation by stimulating soil microbes/microbial consortia with hydrocarbon degradation capabilities in rhizosphere. Phytoremediation is an efficient, conservational and it shows potential technology for removing many contaminants such as hydrocarbon pollutants. The synergy between plant roots and soil microorganisms promotes the degradation of organic contaminants in phytoremediation (Vwioko *et al*., 2012).

This study reports amount of evolved CO_2, effect of available carbonate on various properties, the effects of inorganic fertilizer (NPK agricultural fertilizer) on the microbial utilization of petroleum hydrocarbon on polluted soil in order to determine the component that gives the best performance for remediation purposes.

Microbial status of the soil samples

The evolved CO_2 can be measured and assessed as an index of microbial activity of soil which can be observed in alkali as –

$$2NaOH + CO_2 \Longrightarrow Na_2CO_3 + H_2O$$

The excess of alkali can be found by titrating against HCl

$$NaOH + HCl \Longrightarrow NaCl + H_2O$$

Effect of available carbonate on various properties:

The amount of available carbonates can have a profound effect on physiochemical and biological properties.

Effects of inorganic fertilizer (NPK agricultural fertilizer)

Several concentrations of NPK fertilizer were added to different quantities of polluted soil samples in order to determine the nutrient ratio.

Materials and Methods

Sample collection and preparation

Contaminated soil samples were collected from motor garage areas of Transport Nagar region of Jaipur, Rajasthan. Samples were collected form 5-9 cms of upper surface of contaminated sites. Samples were air dry and pass through 2mm sieve. The processed samples were store in sterile poly beg at room temperature for future testing. The samples were labeled as T1, T2 and T3. And the normal soil (NS) used as control has been collected from the premises of the Rajasthan University, Jaipur.

50 gm of soil sample was weighed and collected in 6 flask to which sterile distilled water was added to 33% of its maximum water holding capacity. 15ml of freshly prepared N/10 NaOH solution was filled in two test tubes, mouth of each test tube was tied and hung in the flask in such a way that free end of the thread remains out of the flask and tubes do not touch the soil surface. Mouth of flask was sealed using rubber stopper and molten wax and were incubated at room temperature.

Each soil sample has weighted 50 gm and added different concentration of Na_2CO_3 and NPK according to the Table1 .

Determination of bicarbonate content (Pandey and Sharma,2003)

Bicarbonate is very important basic ingredient of soil, for its buffering capacity. Bicarbonate can be projected by using its acidimetric titrations in presence of methyl orange as indicator. HCO□ □ presence in the soil is neutralized by the standard acid.

Determination of soil chloride content (Pandey and Sharma,2003)

Chloride can be projected by titration of the soil extract with $AgNO_3$, formation of nearly insoluble salts marks the end of the extraction because chloride is present in the form of soluble salts of sodium, potassium and calcium.

Determination of CO_2 evolved and Carbon Mineralized (Dubey and Maheshwari 2004)

As soil contains lots of microbial flora and live microbes respire and evolve CO_2. For measurement of this evolved CO_2 ,50g of soil sample was weighed and transferred into sterile flasks. Sterile distilled water equal to 33% of its maximum water holding capacity was added to the sample. 15 ml freshly prepared 0.1N NaOH solution was filled into two test tubes were hung in the flask and mouth of flask was packed. Flasks were incubated at room temperature. After incubation of 10 days one flask was taken per 10 day and NaOH was titrated against 0.1N HCL. The enhanced carbon in the mineralization and CO_2 evolution confirmed the increase in bacterial population.

Result and Discussion

After the addition of Na_2CO_3 and NPK the soil respiration increases as compared to normal soil sample. CO_2 evolved and carbon mineralization increases with the increase in NPK and Na_2CO_3 concentration. We used maximum concentration of 40gm of NPK and 40 mg Na_2CO_3. After the incubation period of every 10 days all the parameters were calculated. It was observed that at 60 days of incubation period the maximum soil respiration occur for Na_2CO_3 and NPK with highest at 40 mg concentration. The maximum concentration of $Na_2 CO_3$ shows CO_2 evolved for soil sample T1, T2 and T3 is 30.96, 50.6, 90.64 and carbon mineralization is 8.44, 13.8 and 24.65 respectively. In case of NPK, CO_2 evolved is 46.50, 60.2 and 78.5 and carbon mineralization 12.68, 16.4 and 21.4 respectively. Increase in CO_2 evolution and carbon mineralization shows that increase in the microbial population and enhancement of metabolic activity to utilization of hydrocarbons. The chloride and bicarbonate concentration also increased with the concentration of Na_2CO_3 and NPK.

In conclusion, soil contamination with hydrocarbons has a serious risk to human health and further causes organic pollution of ground water and economic loss, ecological problems and decreases the agricultural efficiency of the soil. The toxicity of petroleum hydrocarbons extends to microorganisms, plants, animals and humans (Margesin *et al*.,2001).This study was done to identify whether agricultural fertilizers (N,P,K) enhances the microbial degradation of hydrocarbons in soil. The results observed showed that application of NPK fertilizers has remarkable effects on bacterial growth (Jain *et al*.,2011). Nitrogen and Phosphorus are necessary for cellular metabolism of microorganisms (Mohan and Steward,2000). Harmonization of C\N\P ratios by addition of assorted nitrogen and phosphorus sources motivate and activate the biodegradation of hydrocarbons in soil (Thapa *et al*.,2012).

Table.1 Shows the different combination of inorganic and agricultural fertilizer NPK with sample soil T1, T2 and T3

S.No.	Additional compound	Amount of additional compound	Sample soil
1.	Na_2CO_3	10mg	50gm
		20mg	50gm
		40mg	50gm
2.	NPK	10gm	50gm
		20gm	50gm
		40gm	50gm

Table.2 Soil Respiration at various concentrations of available carbonates

Days ⟶	10	20	30	40	50	60
CO_2 evolved (control)	15.4	16.02	18.36	19.9	20.6	22.6
T1+10mg Na_2CO_3	15.8	17.6	19.2	21.6	22.4	23.08
T1+20mg Na_2CO_3	18.76	20.18	22.82	23.08	25.56	26.18
T1+40mg Na_2CO_3	25.4	26.32	27.76	28.08	29.04	30.96
T2+10mg Na_2CO_3	16.02	18	20.2	24.6	26.82	27.08
T2+20mg Na_2CO_3	20.6	22.8	28.2	33.8	35.6	36.8
T2+40mg Na_2CO_3	35.4	36.2	37.6	48.8	49.4	50.6
T3+10 Na_2CO_3	18.6	20.18	22.82	23.4	28.56	32.08
T3+20mg	38.5	40.4	42.6	45.4	50.3	58.34

Na_2CO_3						
T3+40mg Na_2CO_3	42.6	43.3	47.4	50.6	59.8	90.64

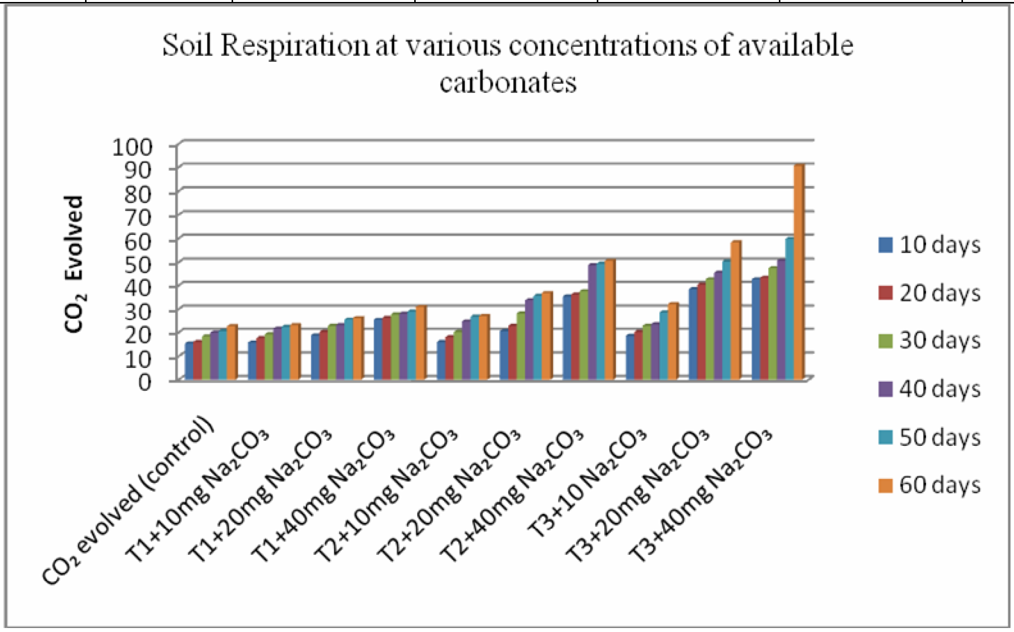


Table.3 Carbon mineralization at various concentrations of available carbonates

Days ⟶	10	20	30	40	50	60
Carbon mineralization (control)	4.18	4.35	4.99	5.41	5.60	6.1
T1+10mg Na_2CO_3	4.29	4.78	5.22	5.87	6.09	6.27
T1+20mg Na_2CO_3	5.10	5.48	6.20	6.27	6.95	7.12
T1+40mg Na_2CO_3	6.90	7.15	7.55	7.63	7.89	8.44
T2+10mg Na_2CO_3	4.35	4.89	5.49	6.69	7.29	7.36
T2+20mg Na_2CO_3	5.60	6.20	7.67	9.19	9.68	10.00
T2+40mg Na_2CO_3	9.62	9.84	10.22	13.27	13.43	13.80
T3+10mg Na_2CO_3	5.05	5.48	6.20	6.36	7.77	8.72
T3+20mg Na_2CO_3	10.47	10.98	11.58	12.34	13.68	15.86
T3+40mg Na_2CO_3	11.58	10.96	12.89	13.76	16.26	24.65

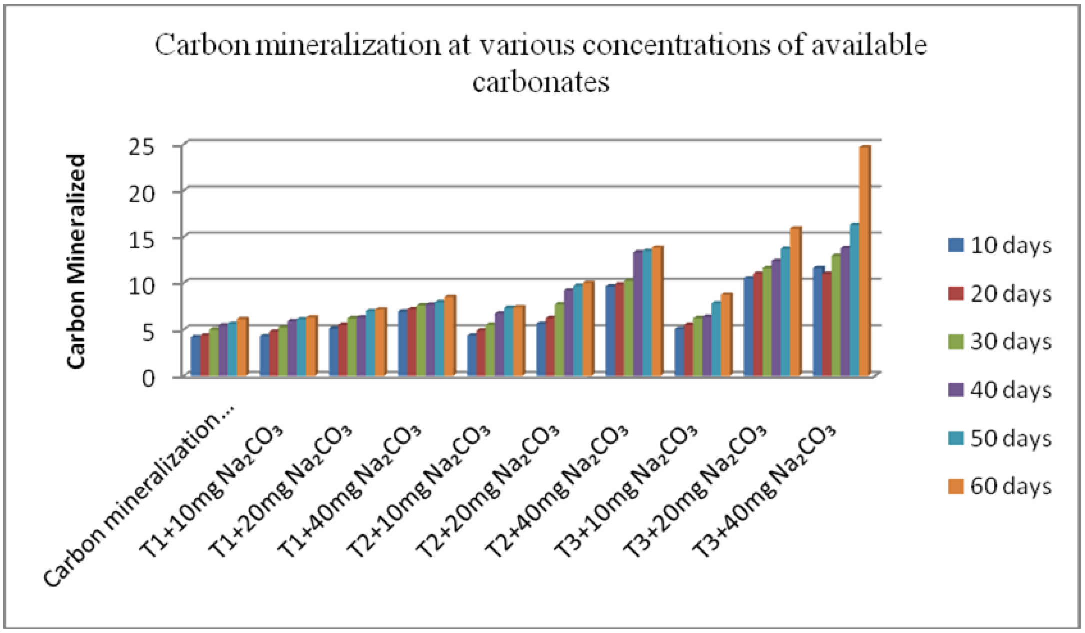


Table.4 pH at various concentrations of available carbonates

Days →	10	20	30	40	50	60
pH (control)	7.63	7.63	8.10	8.10	8.00	7.60
T1+10mg Na_2CO_3	7.80	7.76	7.62	7.48	7.22	7.01
T1+20mg Na_2CO_3	8.20	8.12	7.95	7.76	7.71	7.47
T1+40mg Na_2CO_3	8.90	8.84	8.35	8.28	8.19	7.90
T2+10mg Na_2CO_3	7.92	7.88	7.74	7.53	7.41	7.22
T2+20mg Na_2CO_3	8.32	8.28	7.97	7.63	7.75	7.27
T2+40mg Na_2CO_3	8.21	8.16	8.38	8.49	8.14	7.98
T3+10mg Na_2CO_3	7.03	6.83	6.71	6.32	6.22	6.03
T3+20mg Na_2CO_3	7.20	7.04	6.95	6.72	6.78	6.44
T3+40mg Na_2CO_3	7.90	7.80	7.30	7.20	7.10	6.98

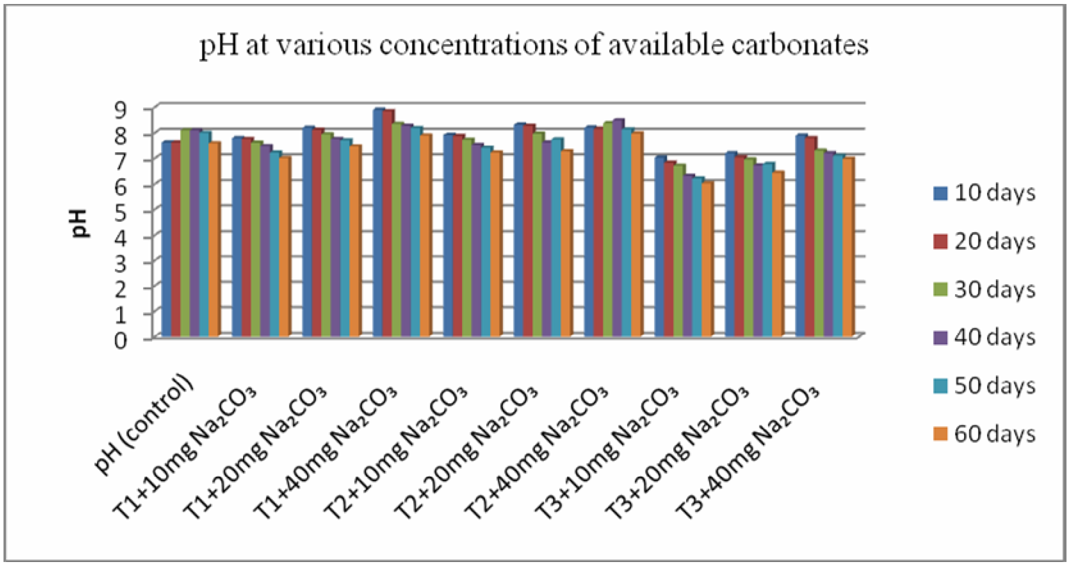


Table.5 Bicarbonate at various concentrations of available carbonates

Days →	10	20	30	40	50	60
Bicarbonate (control)	24.4	26.2	28.2	29.2	30.5	31.5
T1+10mg Na_2CO_3	111.9	114.7	116.5	121.8	128.6	129.5
T1+20mg Na_2CO_3	128.1	129.3	124.9	127.2	129	130.9
T1+40mg Na_2CO_3	135.9	148.1	152.7	164.7	175.9	177.1
T2+10mg Na_2CO_3	102.4	117.8	134.5	154.8	178.9	188.6
T2+20mg Na_2CO_3	118.1	159.3	174.9	197.2	229	230.9
T2+40mg Na_2CO_3	122.9	138.4	152.8	174.7	185.9	197.1
T3+10mg Na_2CO_3	122.3	134.8	143.6	155.2	167.8	172.7
T3+20mg Na_2CO_3	148.1	149.3	156.9	176.2	183.2	190.7
T3+40mg Na_2CO_3	165.1	168.3	172.5	184.7	195.2	207.7

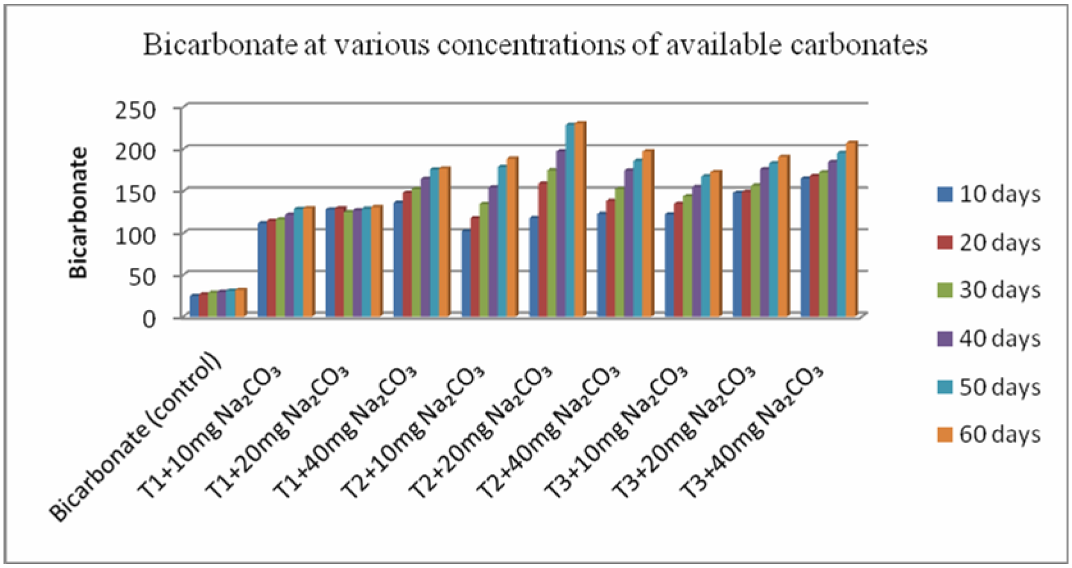


Table.6 Chloride at various concentrations of available carbonates

Days ⟶	10	20	30	40	50	60
Chloride(control)	28.4	29.4	30.8	31.3	32.1	34.2
T1+10mg Na_2CO_3	61.7	64.5	66.7	68.9	70.3	72.3
T1+20mg Na_2CO_3	63.9	69.1	71.0	79.4	81.8	85.5
T1+40mg Na_2CO_3	66.3	75.2	85.2	88.1	95.2	99.7
T2+10mg Na_2CO_3	60.9	64.3	66.8	68.9	70.9	72.4
T2+20mg Na_2CO_3	62.7	65.1	67.8	70.4	71.8	75.5
T2+40mg Na_2CO_3	69.03	77.2	82.2	85.1	91.2	95.7
T3+10mg Na_2CO_3	80.3	84.3	87.8	89.4	91.4	93.4
T3+20mg Na_2CO_3	83.1	89.7	91.3	99.5	101.1	105.5
T3+40mg Na_2CO_3	86.03	95.3	108.4	135.1	195.2	199.7

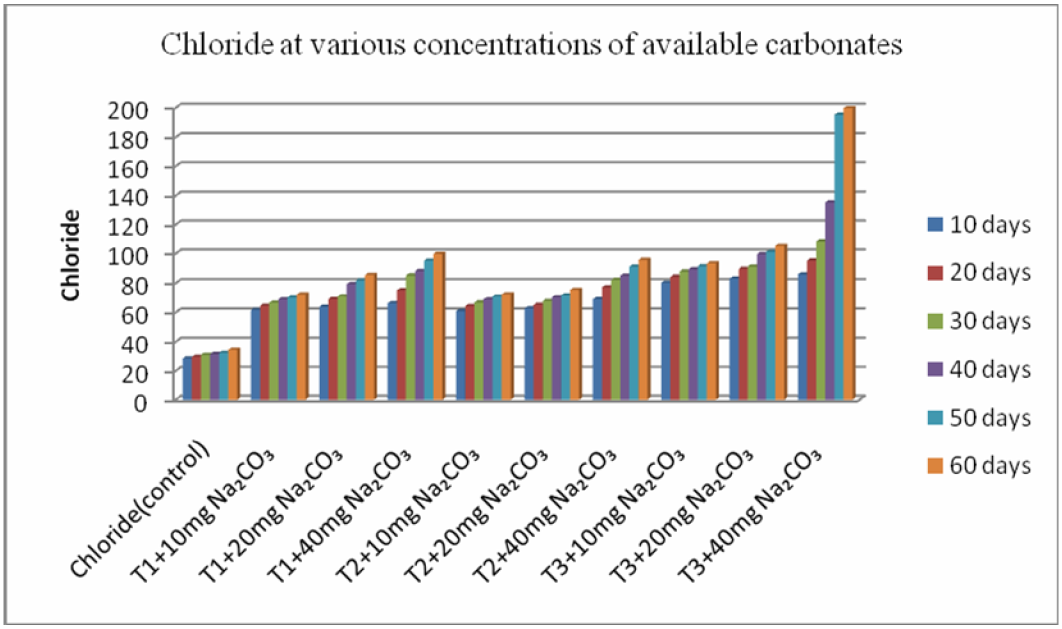


Table.7 Soil Respiration at various concentrations of NPK fertilizers

Days ⟶	10	20	30	40	50	60
CO_2evolved (control)	15.4	16.02	18.36	19.9	20.6	22.6
T1+10gm NPK	20.83	21.76	23.3	24.66	25.55	26.66
T1+20gm NPK	31.32	32.32	33.3	34.1	35.55	36.59
T1+40gm NPK	41.54	42.64	43.32	44.32	45.22	46.50
T2+10gm NPK	22.5	26.7	32.7	36.8	40.2	45.9
T2+20gm NPK	38.3	40.84	42.1	43.84	45.8	46.2
T2+40gm NPK	45.4	56.1	57.4	58.7	59.8	60.2
T3+10gm NPK	25.7	28.4	34.3	42.1	48.2	52.1
T3+20gm NPK	44.2	48.7	52.9	55.7	59.2	61.4
T3+40gm NPK	55.9	59.1	64.3	68.6	73.1	78.5

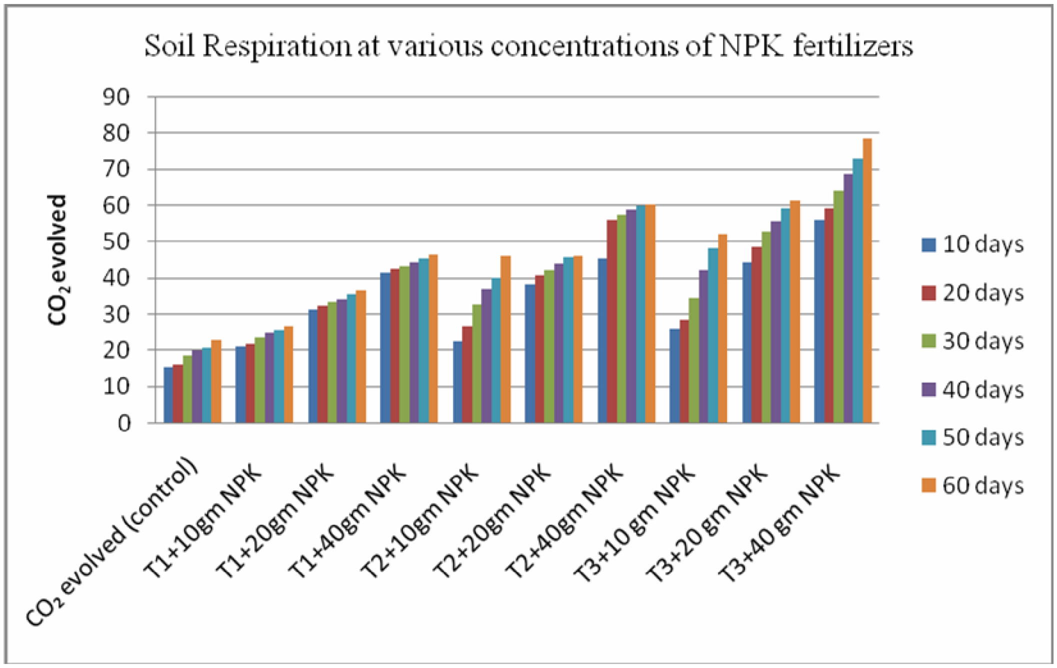


Table.8 Carbon mineralization at various concentrations of NPK fertilizers

Days ⟶	10	20	30	40	50	60
Carbon mineralization (control)	4.2	4.37	5.00	5.42	5.62	6.16
T1+10gm NPK	5.68	5.93	6.35	6.72	6.96	7.27
T1+20gm NPK	8.54	8.81	9.08	9.3	9.69	9.97
T1+40gm NPK	11.32	11.62	11.81	12.08	12.33	12.68
T2+10gm NPK	6.1	7.2	8.9	10.0	10.6	12.5
T2+20gm NPK	10.44	11.1	11.4	11.9	12.4	12.6
T2+40gm NPK	12.3	15.3	15.6	16.0	16.3	16.4
T3+10gm NPK	7.0	7.74	9.35	11.4	13.1	14.2
T3+20gm NPK	12.0	13.2	14.4	15.1	16.14	16.74
T3+40gm NPK	15.2	16.1	17.5	18.7	19.9	21.4

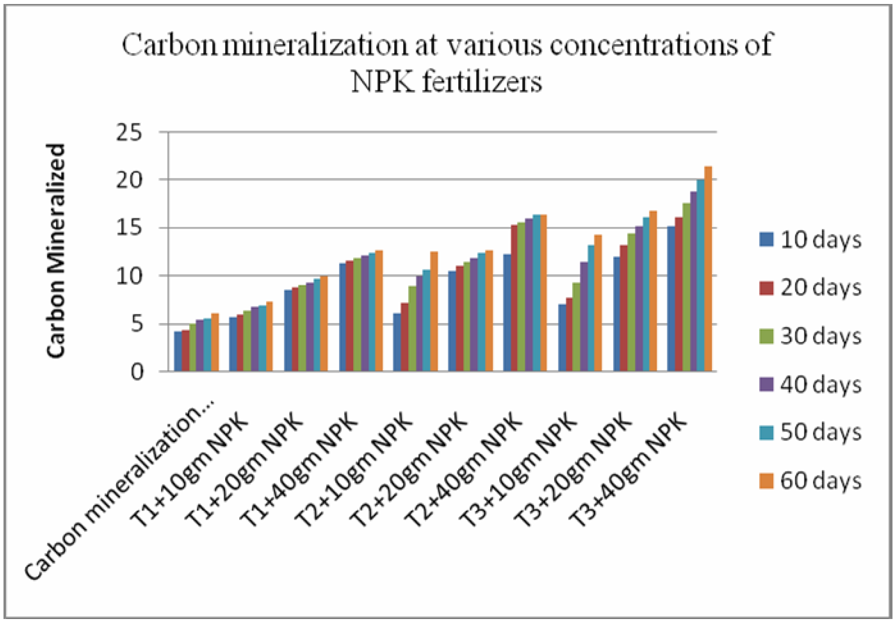


Table.9 pH at various concentrations of NPK fertilizers

Days ⟶	10	20	30	40	50	60
pH (control)	7.63	7.63	8.10	8.10	8.00	7.60
T1+10 gm NPK	6.9	6.7	6.6	6.5	6.3	6.2
T1+20 gm NPK	6.8	6.7	6.7	6.6	6.4	6.3
T1+40 gm NPK	6.7	6.6	6.5	6.3	6.2	6.1
T2+10gm NPK	7.60	7.40	7.2	7.1	7.0	7.0
T2+20gm NPK	7.8	7.6	7.5	7.4	7.1	7.2
T2+40gm NPK	7.90	7.8	7.7	7.6	7.1	7.0
T3+10gm NPK	7.4	7.2	7.2	7.1	7.0	6.9
T3+20gm NPK	6.9	6.7	6.5	6.4	6.3	6.2
T3+40gm NPK	7.3	7.2	7.0	6.8	6.6	6.4

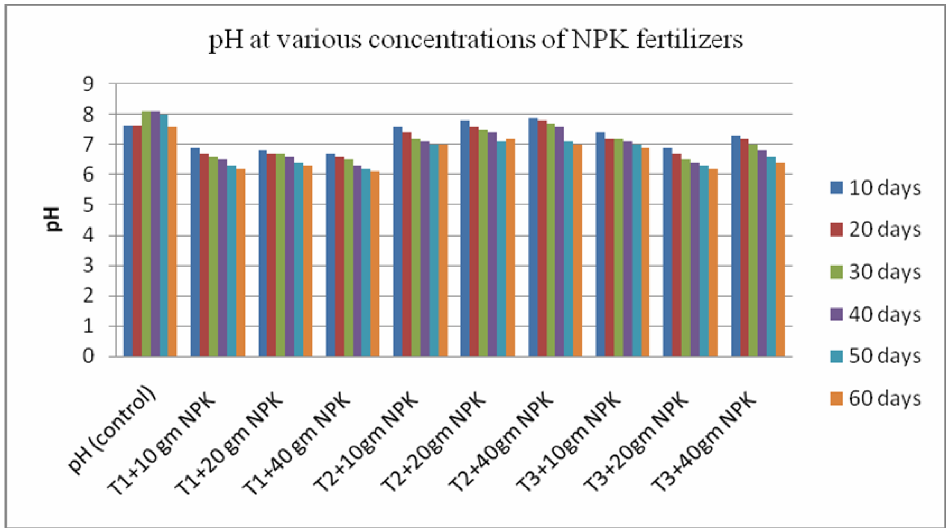


Table.10 Bicarbonate at various concentrations of NPK fertilizers

Days ⟶	10	20	30	40	50	60
Bicarbonate (control)	24.4	26.2	28.2	29.2	30.5	31.5
T1+10 gm NPK	976.9	985.5	1018.7	1274.5	1292.8	1317.2
T1+20 gm NPK	1403.2	1354.3	1426.1	1526.1	1687.1	1768.8
T1+40 gm NPK	1524.6	1673.2	1813.5	2006.5	2118.7	2366.2
T2+10gm NPK	800.2	809.4	815.3	822.4	848.5	867.1
T2+20gm NPK	1509.1	1526.9	1589.7	1621.3	1654.8	1673.2
T2+40gm NPK	1620.3	1634.1	1645.6	1680.2	1685.3	1693.8
T3+10 gm NPK	820.3	829.9	845.8	862.9	888.3	897.9
T3+20 gm NPK	1259.5	1433.1	1761.8	1998.3	2013.2	2215.1
T3+40 gm NPK	1260.1	1432.8	1985.1	2080.3	2385.8	2693.1

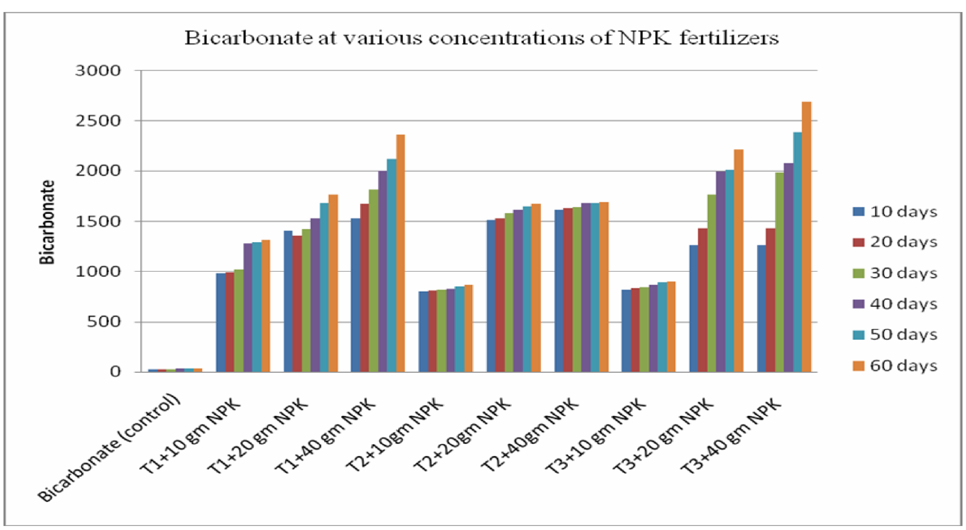
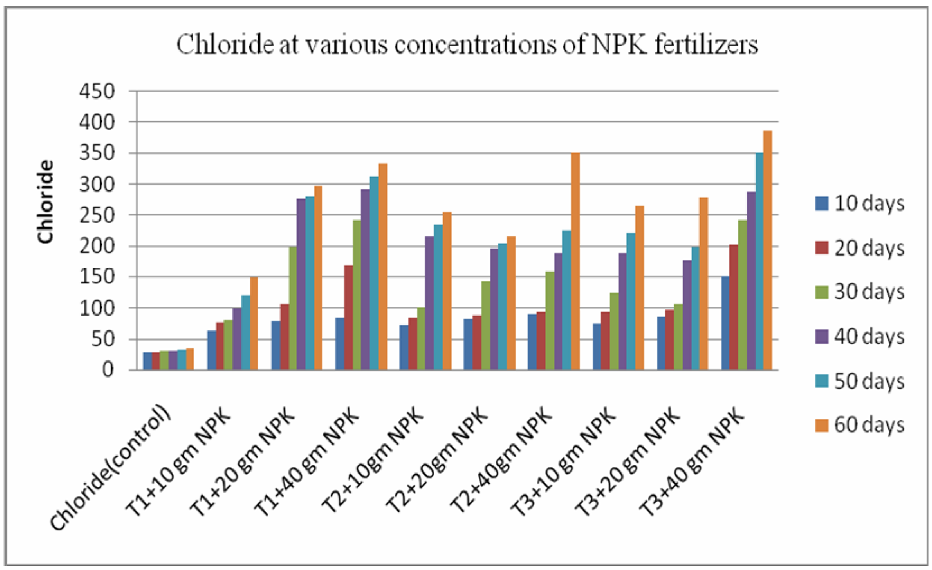


Table.11 Chloride at various concentrations of NPK fertilizers

Days ⟶	10	20	30	40	50	60
Chloride(control)	28.4	29.4	30.8	31.3	32.1	34.2
T1+10 gm NPK	63.3	76.1	80.7	99.4	120.7	149.1
T1+20 gm NPK	78.1	106	198.8	276.5	281.16	298.2
T1+40 gm NPK	83.5	170.4	242.6	291.1	312.4	333.7
T2+10gm NPK	72.3	83.2	101.8	215.9	234.5	256.1
T2+20gm NPK	81.2	86.9	143.4	197.1	203.8	215.2
T2+40gm NPK	88.9	93.2	157.9	188.3	224.6	350.9
T3+10 gm NPK	75.1	93.3	123.7	189.3	221.7	265.8
T3+20 gm NPK	85.8	96.7	106.9	176.4	198.6	278.4
T3+40 gm NPK	150.4	201.4	243.1	288.5	350.3	387.3



References

Anoliefo, G., O. Isikhuemhen and N. Ochije. 2003. Environmental Implications of the erosion of cultural taboo practises in Awka-South local government area of Anambra State, Nigeria: Forests, trees, and water resource preservation. Journal of Agricultural and Environmental Ethics16: 281-296

Bijay Thapa, Ajay Kumar KC, Anish Ghimire (2012) A Review On Bioremediation Of Petroleum Hydrocarbon Contaminants In Soil , Kathmandu University Journal Of Science, Engineering And Technology,8: 164-170

D. E. Vwioko C.E. Omamogho, (2012) Assessing Successive Plant Growth On Petroleum Hydrocarbon Degradation In Highly Polluted Soil Augmented With Wood Ash, International Journal Of Applied Science And Technology, 2:247-267

Dubey, R.C. and Maheshwari, D.K. 2004. Practical Microbiology, (S.chand and company Ltd, New Delhi).

Esteban G. Jobba´ Gy, And Robert B. Jackson, (2000) The Vertical Distribution Of Soil Organic Carbon And Its Relation To Climate And

Vegetation, Ecological Applications, 10, 423–436

J. Curiel Yuste , D. D. Baldocchi , A. Gershenson , A. Goldstein , L. Misson And S . W O N G , (2007) Microbial Soil Respiration And Its Dependency On Carbon Inputs, Soil Temperature And Moisture, Global Change Biology 13, 1–18

Killops S. D. and Al-Juboori M. A. H. A. (1990) Characterisation of the unresolved complex mixture (UCM) in the gas chromatograms of biodegraded petroleums Org. Geochem15, 147–160.

M.Chorom, H.S. Sharifi, H. Motamedi (2010) Bioremediation of crude oil-polluted soil by application of fertilizers, 7:319-326

Pandey J. and M.S. Sharma (2003). Analysis of soil. P.37-53. In Environmental Science, Practical and Field Manual. Yash Publishing House.

Pankaj Jain, Abhishek Vashishtha, Madan Lowry and D.P. Jaroli (2011) , Chapter 5: Hydrocarbon Bioremediation: Biotic And Abiotic Factors, Recent Advances in Environmental Biotechnology, 112-145

R. Margesin , F. Schinner (2001) Biodegradation and bioremediation of hydrocarbons in extreme environments, Appl Microbiol Biotechnol, 56:650–663

Snežana Maletić, Božo Dalmacija and Srđan Rončević (2013) Petroleum Hydrocarbon Biodegradability in Soil – Implications for Bioremediation, INTECH, 43-64