

Full Length Research Paper

Assessment of the impact of kerosene and diesel on soil nitrogen mineralisation

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Accepted 9th November, 2010

Effects of different concentrations (1, 2 and 5% v/w) of diesel and kerosene on N mineralization were studied in a sandy loam soil. Relative to the untreated soil, higher ammonium-N levels were obtained in samples treated with all the concentrations of kerosene and diesel from the second and third weeks of incubation respectively while extents of nitrate-N production differed. Net N-mineralization was 17.211 mg mineral-N/kg soil in untreated soil and -11.753 mg mineral-N/kg soil and -26.562 mg mineral-N/kg soil respectively in soils treated with 5% kerosene and diesel after six weeks incubation. Net nitrification was 29.90 mg NO₃-N/kg soil in untreated soil and -9.35 mg NO₃-N/kg soil and -22. NO₃-N/kg soil respectively in soils treated with 5% kerosene and diesel after six weeks incubation. Overall mineralization rates were highest (13.01 mg N/kg soil per week) in untreated soil. Rates were lower in diesel than in kerosene treated soils. Inhibition of net N mineralisation ranged from of 139.31 to 168.29% and 90.41 to 254.34% after sixth week incubation for kerosene and diesel-treated soils respectively. The toxicity of diesel and kerosene treatment on nitrogen mineralization in the soil is due more to their effects on ammonium-N than on nitrate-N production.

Key words: Mineralisation, nitrification, ammonium, nitrate, diesel and kerosene.

INTRODUCTION

Nitrogen is a key element in many compounds of plant cells and its availability for crop plants is an important limiting factor for crop production on a global basis (Foth and Ellis, 1997). In soil, the bulk of nitrogen is present in the gaseous or organic form, however, plants use nitrogen almost entirely in the inorganic form. Through biological nitrogen fixation, atmospheric nitrogen is converted to inorganic nitrogen (NH₃), and through nitrogen mineralization, organic nitrogen is converted to inorganic forms (ammonium and nitrate). Both nitrogen fixation and mineralization are brought about by the activities of soil microorganisms. In mineralization, organic nitrogen is first converted to ammonium (NH₄⁺) through the process of ammonification and through nitrification, NH₄⁺ is converted to nitrate (NO₃⁻) a more mobile form. Apart from nitrification, NH₄⁺ once in soil solution can be held as an exchangeable cation on negatively charged surfaces as found on some soil clay particles, lost by conversion to gaseous ammonia (NH₃)

under alkaline soil conditions, or assimilated by soil microorganisms and plants to supply their N requirements; through the process of immobilization (Deenik, 2006). Through immobilization, soil inorganic N is assimilated back into the microbial population and is tied up as organic N, temporarily reducing the plant-available N pool, and through nitrification, NH₄⁺ is converted to a more mobile form (NO₃⁻). Thus immobilization, nitrification and ammonification are vital processes that regulate plant available nitrogen in soil.

In terrestrial ecosystems, N mineralization is affected mainly by climate, soil physical and chemical properties and the quality and quantity of soil organic matter (Aubert et al., 2005). Pollution of arable lands by hydrocarbons arising from transportation, exploration and exploitation of crude oil and its refined derivatives affect soil properties in various ways. In their investigation, Deni and Penninckx, (1999) observed that addition of hydrocarbon to an uncontaminated soil stimulated immobilization of nitrogen derived from mineralization of the available organic nitrogen, leading to reduced nitrification. Several *in vitro* studies have shown that pure cultures of *Nitrosomonas europaea*, a ubiquitous ammonium

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oxidiser also oxidize a wide variety of hydrocarbon substrates through the action of ammonia monooxygenase the first key enzyme in the autotrophic nitrification process (Hyman et al., 1998). Such co-metabolism of hydrocarbons via the ammonia monooxygenase pathway may be considered a competitive cooxidation which reduces the rate and extent of ammonia oxidation. Okpokwasili and Odokuma (1996) reported that pure cultures of *Nitrobacter* sp tolerated diesel more than kerosene and jet fuel. Such pure culture studies ignore interactions of bacteria and environmental components. This study investigates the effects of diesel and kerosene on soil N transformation processes. Measuring the net change in N concentration is the most widely practiced method of assessing N transformations especially with respect to plant nutrition (Habteselassie et al., 2006). Such a study is necessary because information concerning nitrogen transformation processes that occur in the presence of hydrocarbon in a natural soil medium helps in developing effective bioremediation strategies in case a spillage occurs.

MATERIALS AND METHODS

Sample collection and preparation

Prior to treatment, soil samples were randomly collected at 0 to 5 cm soil depth from an agricultural farm into a sterile plastic bag and delivered to the laboratory within one hour. The samples were homogenized, air-dried, sieved through 2 mm mesh and then stored in a polythene bag.

Soil characterization

Sub-samples of the fine earth fractions were analyzed for physicochemical and microbiological properties. Particle size distribution was analyzed by the hydrometer method (Gee and Bauder, 1986); pH in 1:2.5 soil/water ratio (McLean, 1982); organic carbon by the modified wet combustion method (Nelson and Sommers, 1982); total nitrogen by the semi-micro Kjeldhal method (Bremner and Mulvaney, 1982), exchangeable sodium and potassium by flame photometry, ammonium-nitrogen by the nesslerisation method (Keeney and Nelson, 1982) and nitrate-nitrogen by the phenoldisulphonic acid method (Bremner, 1965).

Soil microbial population was estimated by the ten-fold serial dilution method of Harrigan and McCance (1990). Population of total heterotrophic bacteria was estimated using nutrient agar (Oxoid) while those of the ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) were estimated using the modified medium of Schmidt and Belsar (1999). Plates were incubated at room temperature ($28 \pm 2^\circ\text{C}$) for 24 to 48 h for heterotrophs and for 9 to 14 days for the AOB and NOB.

Incubation study

About two hundred grams of soil samples was weighed into plastic containers (8.3×6.3 cm), moistened to about 50 to 60% field capacity and covered with perforated cellophane sheets (to prevent moisture loss and ensure adequate aeration). The containers were then stored at room temperature ($28 \pm 2^\circ\text{C}$) in the laboratory, for one week. Subsequently, the containers were treated with 0.075 g of

NPK fertilizer (20:10:10), covered and left at room temperature ($28 \pm 2^\circ\text{C}$) for another one week. Thereafter, treatments equivalent to 1.0, 2.0 and 5.0% v/w concentrations of kerosene and diesel were added, covered with perforated cellophane and at room temperature ($28 \pm 2^\circ\text{C}$) in the laboratory for further five weeks. During this period, moisture loss obtained by difference was maintained using deionised water and sub-samples (about 5 g) taken from each container, at weekly intervals (0 to 6 weeks). Sub-samples were air dried and analyzed for ammonium and nitrate. Also samples were analysed for organic carbon and total nitrogen at the end of the sixth week incubation using the methods described above.

Determination of Ammonium-N and Nitrate-N

The ammonium-N and nitrate-N in soil samples were determined spectrophotometrically from the samples after extraction with Morgan's extracting solution as described by Keeney and Nelson (1982). About 7.5 g of the air-dried soil samples and 0.35 g of activated charcoal were weighed into 30 ml plastic containers and 28 ml of Morgan's extracting solution added. The containers were shaken on a reciprocating shaker for one hour and filtered into 50 ml conical flasks using Whatman (No.1) filter paper.

Ammonium-N in samples were determined by pipetting a 5 ml of the supernatant into a 15 ml test-tube and adding 2.4 ml sodium potassium tartarate, 0.8 ml alkaline sodium phenate, 0.8 ml sodium hypochlorite and 2 ml deionized water into the test tube. The contents of the tubes were thoroughly mixed and left to stand for about 20 min at room temperature ($28 \pm 2^\circ\text{C}$). $\text{NH}_4\text{-N}$ content in the solution was determined on a Spectronic 20 spectrophotometer at a wavelength of 630 nm.

Similarly, nitrate-N in samples were determined by pipetting 5 ml of the supernatant into a 15 ml test-tube and adding 0.8 ml brucine, 4 ml concentrated H_2SO_4 and 1 ml deionized water. Absorbance was read at 470 nm on a Spectronic 20 spectrophotometer.

Estimation of net N mineralization

The difference between final and initial levels of total inorganic N (ammonium-N + nitrate-N) is net N mineralization. The net N mineralization, nitrification and ammonium consumption rates were calculated as the change in the inorganic N or nitrate-N pool size or ammonium-N consumed respectively.

The overall rate of mineralization was calculated from the slope of the linear regression curve of mineral-N formed versus time. The relative effect of the different concentrations of kerosene on net N mineralization was calculated from absolute mineralization values using Formula (1):

$$a = \left(\frac{b-c}{b} \right) \times 100 \quad (1)$$

Where:

- a = Relative effects on net mineralisation at time t.
- b = Net mineralisation in control at time t.
- c = Net mineralisation in treatment at time t.

RESULTS AND DISCUSSION

The physicochemical properties of the treated and untreated soil (Table 1) showed that the untreated soil

Table 1. Some properties of treated and untreated soil samples.

Properties	Untreated soil	Treated soil					
		Kerosene (% ^v /w)			Diesel (% ^v /w)		
		1.0	2.0	5.0	1.0	2.0	5.0
Organic carbon (%)	1.821	2.1746	2.3732	2.6166	2.5137	2.7300	3.1905
Total nitrogen (%)	0.116	0.140	0.143	0.145	0.143	0.145	0.152
C:N ratio	18:1	18:1	19:1	21:1	21:1	22:1	24:1
Organic matter (%)	3.139						
pH in H ₂ O	5.62						
Exchangeable base (Meq/100 g)							
Ca	0.600						
Mg	0.250						
Na	0.022						
K	0.042						
P	4.2						
Exchangeable acids(Meq/100 g)							
Al	0.320						
H	0.480						

was a sandy loam soil with a pH of 5.62; the percentage organic carbon was 1.821, percentage organic matter was 3.139%, and total nitrogen was 0.116%. Treatment with the different hydrocarbon fractions increased the C:N ratios progressively with the level of treatment because kerosene and diesel are rich in carbon but deficient in nitrogen. Solano-serena et al. (2000) also reported increased C:N ratio following hydrocarbon contamination of soil.

The microbiological analysis showed that the populations of ammonium oxidizing bacteria, nitrate oxidizing bacteria and total heterotrophic bacteria in the untreated soil were 2.08×10^6 , 1.64×10^6 and 4.03×10^6 cfu/ml respectively. Mineralisation studies showed that ammonium-N levels decreased progressively in the untreated soil throughout the six weeks of incubation (Figure 1), however treatment with either kerosene or diesel affected ammonium-N levels in different ways. Ammonium-N levels were higher in samples treated with all the concentrations of kerosene and diesel than in the control from the second and third weeks of analysis respectively. Higher ammonium-N levels could indicate enhanced ammonification, reduced ammonium immobilization and reduced nitrification since these processes are intricately linked in soil and could influence the level of ammonium in soil (Deenik, 2006). The changes in soil nitrate-N levels (Figure 2) showed that concentrations increased progressively in the untreated soil while the patterns with treatment rates varied for both kerosene and diesel. When applied at 5% (v/w) progressive reductions in nitrate-N production were recorded with both kerosene and diesel. With kerosene, at 1.0 (v/w) slight increase in nitrate-N level was

observed up till the fourth week of analysis after which nitrate-N level reduced while at 2% (v/w) there was no significant change in nitrate level throughout the incubation period. With diesel, nitrate-N level dropped in the first two weeks with 1% (v/w) and three weeks with 2 and 5% (v/w), and increased thereafter. Overall, relative to the control, nitrate-N production decreased in soils treated with all the concentrations of kerosene and diesel. Deni and Pennickx (1999) also reported lower increase in nitrate production following addition of diesel to an agricultural soil. A possible explanation for this might be a competition for ammonia monooxygenase, the first key enzyme in the autotrophic nitrification process which has been found to also cometabolise hydrocarbons (Hyman et al., 1998). This implies that reduced nitrification contributed to the higher ammonium-N levels observed in the treated soils. The decreasing ammonium-N levels as well as the increasing nitrate levels observed in the control with time is evidence of nitrification in a soil that is free from contamination. This is further accentuated by a strong negative correlation observed between the levels of nitrate-N and ammonium-N in the control (-0.99) and the weak negative (-0.17) or strong positive correlation (0.99) between both parameters in the variously treated soils.

Estimation of net nitrification and N mineralization in the untreated and the variously treated soils (Tables 2 to 3) showed a progressive increase in net nitrification and nitrogen mineralization in the control throughout the incubation period. The total nitrate-N and mineral-N contents in the control were 29.90 mg NO₃-N/kg soil and 17.211 mg mineral N/kg soil after six weeks incubation. Net nitrification and N mineralization values were positive

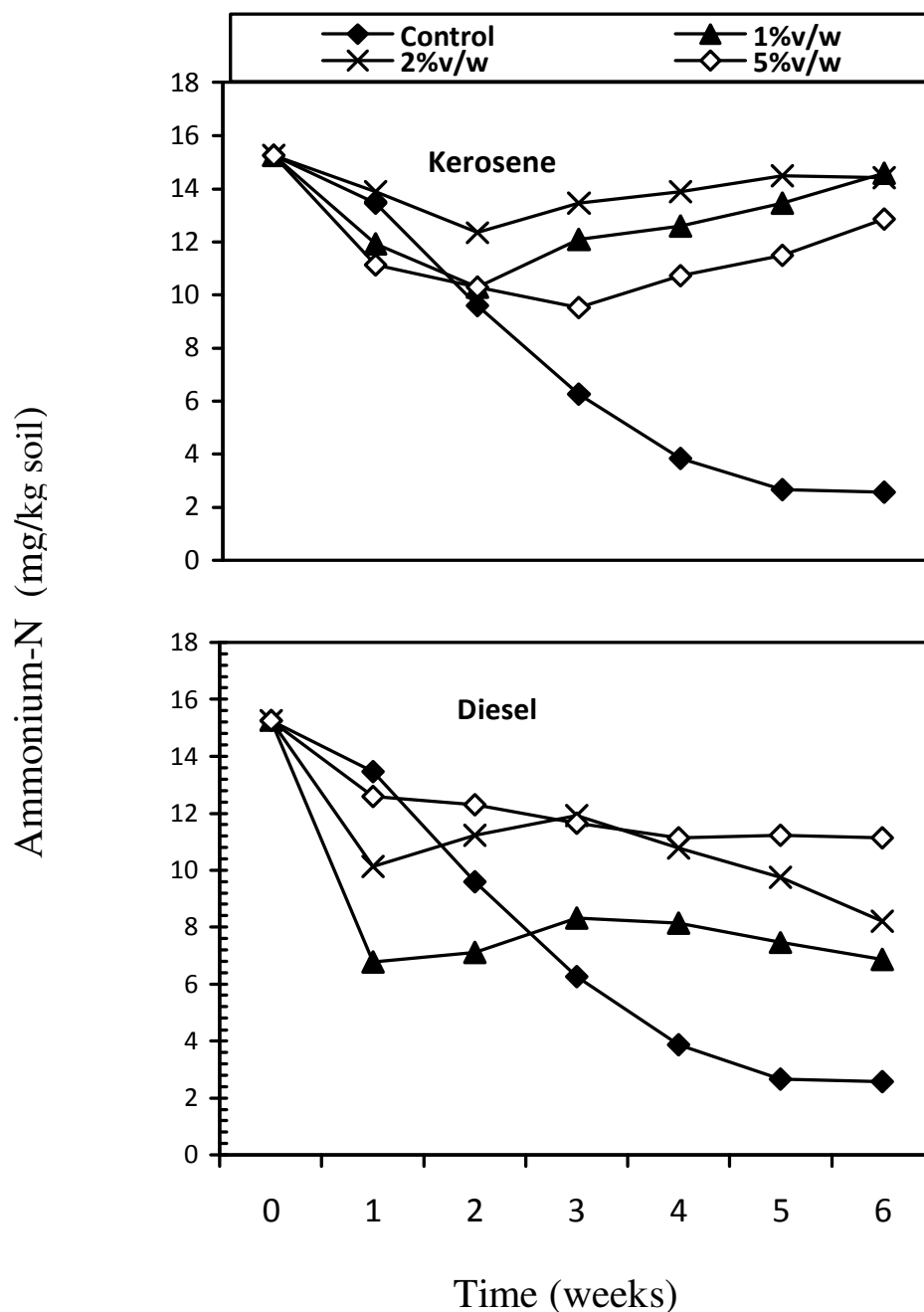


Figure 1. Changes in the concentrations of ammonium-N in soil given different concentrations of kerosene and diesel.

in soils treated with 1% (v/w) kerosene up to four weeks of incubation and negative in soils treated with 2 and 5% (v/w) kerosene and diesel throughout the incubation periods. Negative net mineralization values indicate immobilization and a depletion of soil mineral nitrogen (Hart et al., 1994).

The resultant increase in C:N ratio accompanying treatment of soil with diesel and kerosene must have led to the selective enrichment of diesel and kerosene

utilizing microbes. There may also have been a concomitant increase in other heterotrophic microbes, thus creating a short-term increase in biomass (Morgan and Watkinson, 1989), which could have mopped up the available mineral nitrogen for cell use and biomass increase. Bossert and Bartha (1984) also reported immobilization of soil nitrogen following oil pollution. For kerosene, treatment at 1% (v/w) which did not appreciably increase the C:N ratio of the soil did not

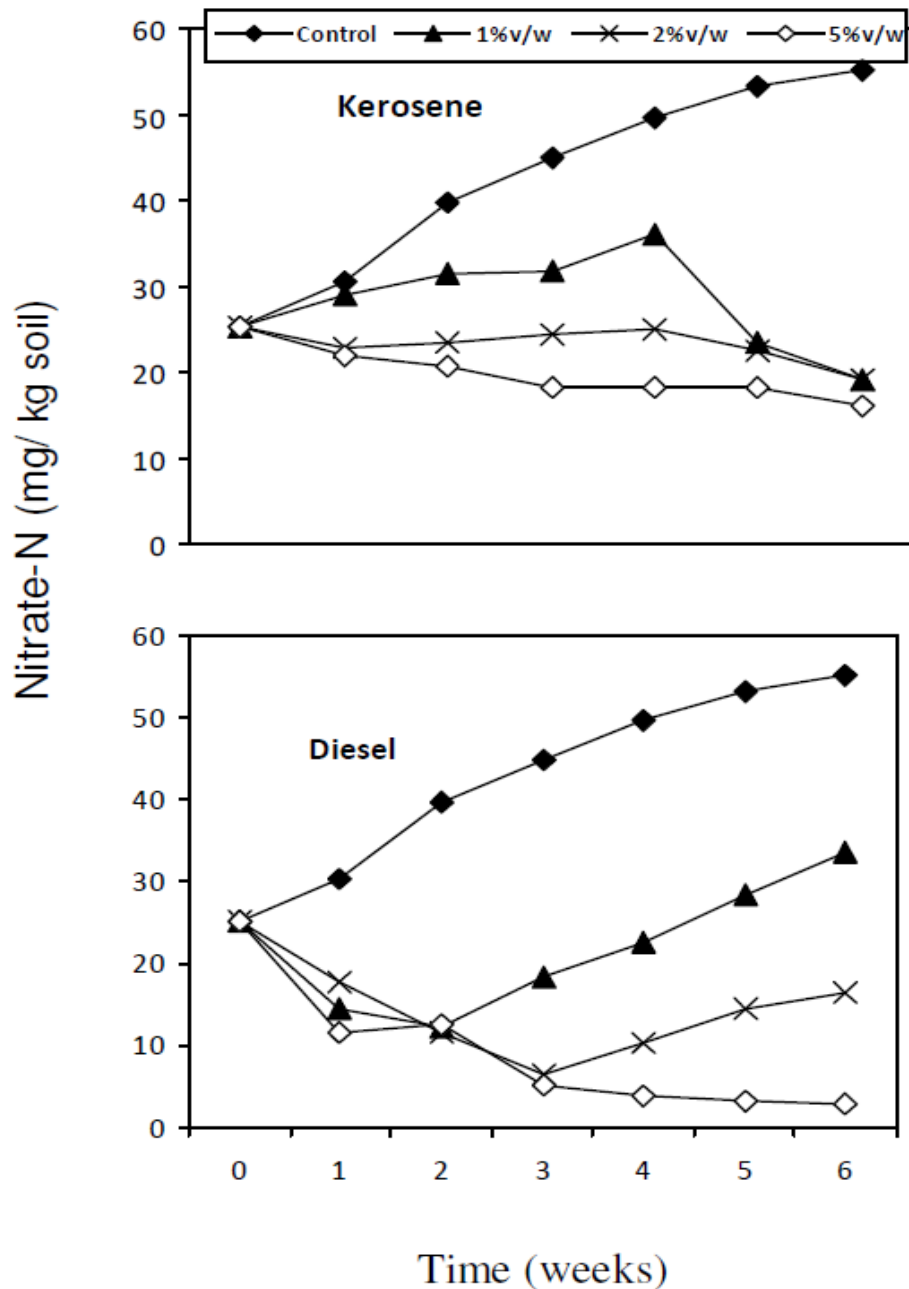


Figure 2. Changes in the concentrations of nitrate-N in soil given different concentrations of kerosene and diesel.

adversely affect nitrification and N mineralization until after four weeks incubation and the inhibition imposed by 1% (v/w) diesel was transient since this was alleviated after four weeks incubation. This might represent differences in the chemical composition of kerosene and diesel.

The overall mineralization rate (Table 4) deduced from the slope of a plot of cumulative nitrogen mineralized versus time (plot not shown) for treatments which fitted a

straight line graph of the equation $y = mx + c$ with R^2 values ≥ 0.87 , showed that the control had the highest mineralization rate of 13.01 mg/kg soil mineral-N per week. Mineralization rates decreased with increasing concentration of kerosene and diesel. At 5% concentration of kerosene and diesel, the rates were -11.41 and -23.970 mg/kg soil mineral-N per week respectively. This is not surprising since the rapidness of nitrogen mineralization from organic compounds is a

Table 2. Net nitrification in soils given different concentrations of kerosene and diesel.

Time (week)	Nitrate - nitrogen (mg/kg soil)						
	Kerosene (% v/w)				Diesel (% v/w)		
	Control	1.0	2.0	5.0	1.0	2.0	5.0
1	5.15	3.74	-2.34	-3.27	-10.75	-7.48	-13.56
2	14.50	6.08	-1.87	-4.67	-13.09	-13.56	-12.62
3	19.64	6.55	-0.93	-7.01	-7.01	-18.70	-20.11
4	24.32	10.76	-0.47	-7.01	-2.80	-14.96	-21.51
5	28.06	-1.87	-2.80	-7.01	3.28	-10.75	-21.98
6	29.90	-6.08	-6.08	-9.35	8.42	-8.88	-22.44

Table 3. Net N-mineralisation in soils given different concentrations of kerosene and diesel.

Time (week)	Mineral- nitrogen (mg/ kg soil)						
	Kerosene (% v/w)				Diesel (% v/w)		
	Control	1.0	2.0	5.0	1.0	2.0	5.0
1	3.344	0.398	-3.710	-7.388	-19.243	-12.626	-16.219
2	8.839	1.107	-4.785	-9.649	-21.238	-17.591	-15.578
3	10.639	3.375	-2.736	-12.758	-13.959	-22.049	-23.709
4	12.915	8.098	-1.839	-11.558	-9.921	-19.455	-25.626
5	15.456	-3.671	-3.577	-10.787	-4.528	-16.272	-26.008
6	17.211	-6.765	-6.937	-11.753	0.016	-15.939	-26.562

Table 4. Overall rates of mineralization in soils treated with kerosene and diesel.

Concentration (%v/w)	Mineral-N (mg/kg soil per week)	
	Kerosene	Diesel
Control	13.010	13.010
1	4.11*	-9.810
2	-3.59	-18.550
5	-11.41	-23.970

* Overall rate was calculated for only week 1-4 which was linear.

function of the carbon:nitrogen ratio (C:N) of the material (Camberato, 2001) which the various treatments imposed on the soil.

At any given concentration, overall mineralization rates were lower in diesel than in kerosene treated soils. This implies that spills of diesel in soils have a more adverse effect on N-mineralisation than those of kerosene. Hence the use of organic N sources in bioremediation strategies will likely be more effective in kerosene than in diesel spills since mineralization of the organic-N is a prerequisite to its further utilization by either plants or microbes. This is in contrast with earlier reports by Okpokwasili and Odokuma (1996) that *Nitrobacter* a nitrite oxidiser tolerated diesel more than kerosene. This discrepancy might be due to the fact that the study by Okpokwasili and Odokuma (1996) was done with a pure culture while the present study was done with whole soil. Very often, pure culture studies do not truly represent natural environmental conditions since such studies ignore interactions of bacteria and environmental

components and bacterial diversity (Stephen et al., 1998).

The extent of inhibition of mineralization (Figure 3) showed that at the sixth week, higher concentrations of diesel were significantly more inhibiting than lower concentrations while there were no significant differences in inhibition with kerosene concentrations of 1 to 5% (v/w). After six weeks of incubation of soil treated with 5% v/w of either kerosene or diesel, the extents of inhibition of N- mineralisation were 138.142 and 190.029% respectively. Nitrogen mineralization is a measure of soil quality. The decline in N mineralization indicates low microbial activity and a degradation of the biological properties of the soil (Deenik, 2006). Low levels of mineral N in kerosene and diesel treated soils indicates a paucity of mineral N in the treated soils and the obvious implication of the unsuitability of such soils for agricultural purposes. The contributions of the overall processes of ammonium production and nitrate production to net N mineralization in the soils were estimated by assessing

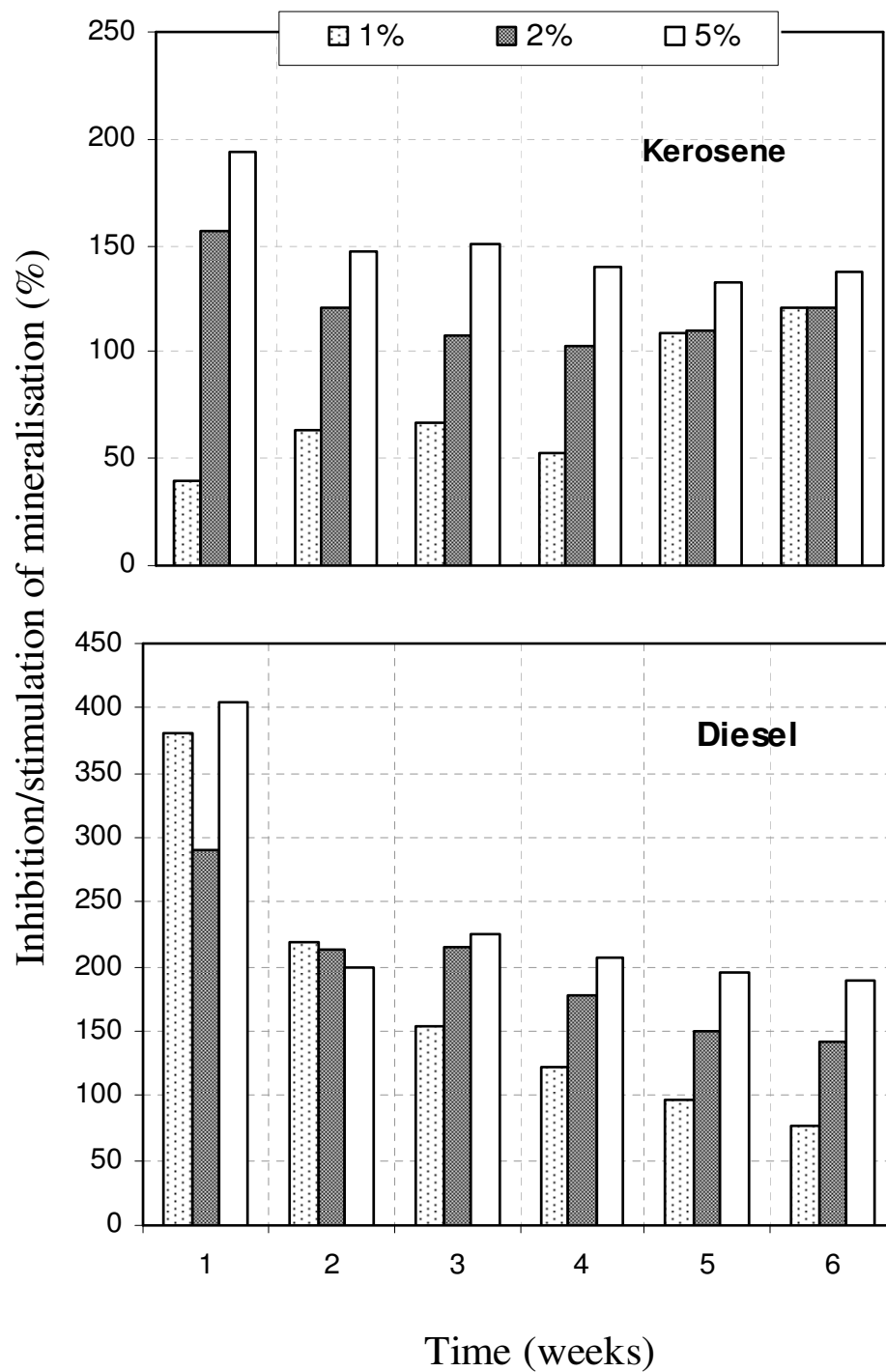


Figure 3. Relative effects of different concentrations of kerosene and diesel on net nitrogen mineralisation in soil.

the relationships between ammonium-N consumption, nitrification and net mineralization rates. Results showed that for both the untreated and the diesel and kerosene treated soil, the relationships between mineralization and nitrification rates were strong and positive ($1 > r > 0.75$).

The patterns of net N mineralization and nitrification rates for an individual treatment are very similar, indicating that the mineralized ammonium was quickly nitrified. Thus the limiting factor to N mineralization in the treated soil was the availability of ammonium. On the contrary,

ammonium-N consumption rate and net N mineralization rates were strongly and positively correlated in the control (0.92) but were either weakly positive or strongly negatively correlated ($0.24 > r > -0.98$) in the treated soils. This implies that in the control, all ammonium-N produced through mineralization were quickly consumed while this is not the case in the treated soils. The rates of ammonium-N consumption in the treated soils were higher than rate of production. Thus the effects of diesel and kerosene treatment on nitrogen mineralization in the soil are due more to their effects on ammonium-N production than on nitrate-N production. This observation reflects the deficiency of organic nitrogen in kerosene and diesel depicted in the increased C: N ratios of the treated soils.

Conclusion

Findings from this work indicate that the toxic effects of diesel and kerosene on soil N mineralisation is due more to their effects on ammonium-N than on nitrate-N production. Ammonium-N supply therefore seems to be the limiting factor for N mineralisation in kerosene and diesel treated soils.

REFERENCES

- Aubert M, Bureau F, Vincelas-Akpa M (2005). Sources of spatial and temporal variability of inorganic nitrogen in pure and mixed deciduous temperate forest. *Soil Biol. Biochem.* 37: 67-79.
- Bossert I, Bartha R (1984). The fate of petroleum in soil ecosystem. In *Petroleum Microbiology*. (Atlas, R.M. ed). Macmillan Publishing company, New York, p. 434-476.
- Bremner JM (1965). Total Nitrogen. In *Methods in Soil Analysis: Chemical and Microbiological Properties Part II*. C.A. Black (ed) Amer. Soc. Agron., p. 1149-1178.
- Bremner JM, Mulvany CS (1982). Total nitrogen determination. In *Method of Soil Analysis vol. 2*, A.L. Page, R.H. Miller and D.R. Keeney (eds) Am. Soc. Agron., p.595.
- Camberato JJ (2001) Nitrogen in Soil and Fertilizers. *Turfgrass Foundation News*, 8: 6-10.
- Deenik J (2006). Nitrogen mineralization potential in important agricultural soils of Hawai'i. *Soil and Crop Management*. 15: 1-5
- Deni J, Penninckx J (1999) Nitrification and Autotrophic Nitrifying Bacteria in a Hydrocarbon-Polluted Soil. *Appl. Environ. Microbiol.*, 65: 4008-4013
- Foth HD, Ellis BG (1997). *Soil fertility*. CRC Press, Boca Raton, FL.
- Gee GW, Bauder JW (1986). Particle-size analysis. p. 383-411. In A. Klute (ed.) *Methods of soil analysis. Part 1. Physical and mineralogical methods*. American Society of Agronomy, Soil Science Society of America, Madison, WI.
- Habteselassie MY, Stark JM, Miller BE, Thacker SG, Norton JM (2006). Gross nitrogen transformations in an agricultural soil after repeated dairy-waste application. *Soil Sci. Soc. Am. J.*, 70: 1338-1348
- Harrigan WF, McCance MC (1990). *Laboratory Methods in Food and Dairy Microbiology*. Academic Press, London.
- Hart SC, Stark MJ, Davidson EA, Firestone (1994). Nitrogen Mineralization, Immobilization and Nitrification. In *Methods Of Soil Analysis 1. part 2. Microbiological and Biochemical properties*. Ed Weaver RW, Angle S, Bottomley P, Bezdak D, Smith S, Tabatabai A, Wolhem A, Michelson SH, Bigham JM, Soil Science Society of America Madison, USA. pp 985-986.
- Hyman MR, Murton IB, Arp JD (1998). Interaction of ammonia monoxygenase from *Nitrosomonas europaea* with alkanes, alkenes, and alkynes. *Appl. Environ. Microbiol.*, 64: 3187-3190.
- Keeney DR, Nelson DW (1982). Determination of mineral (inorganic) nitrogen. In *Methods of Soil Analysis. Vol 2*. (A.L Page, R.H. Miller and D.R.Keeney, eds). Am. Soc. Agron. Madison, p. 643
- McLean EO (1982). Soil pH and lime requirement. In *Methods of Soil Analysis. Part 2. Chemical and Microbiological properties 2nd ed*. Am. Soc. Agron., Inc., Madison, Wisconsin.
- Morgan P, Watkinson RJ (1989). Hydrocarbon degradation in soils and methods for soil biotreatment. *CRC Critical Reviews in Biotechnology* 8: 305-328.
- Nelson DW, Sommers LE (1982). Determination of organic carbon. In *Method of Soil Analysis vol 2*. A. L. Page, R.H. Miller and D.R. Keeney (eds) Am. Soc. Agron. Madison, p. 539.
- Okpokwasili GC, Odokuma LC (1996). Tolerance of *Nitrobacter* to toxicity of hydrocarbon fuels. *Journal of Petroleum Science and engineering* 16:89-93
- Schmidt, E.L. and Belser, L.W. (1999). Autotrophic nitrifying bacteria. In *Methods of soil Analysis 1 part 2 – microbiology and biochemical properties*. In Weaver, R.W., Angle, S., Bottomley, P., Bezdak, D., Smith, S., Tabatabai, A., Wolhim, A., Mickelson, S.H., Bigham, J.M. (editors). Soil Science Society of America. Madison Wisconsin, U.S.A. pp 159-177.
- Solano – Serena F, Marchal R, Lebeault JM, Vacndee casteel JP (2000). Selection of microbial populations degrading recalcitrant hydrocarbons of gasoline by culture head space monitoring. *Lettes in Appl. Microbiol.*, 30: 19-22.
- Stephen JR, Kowalchuk GA, Bruns MV, McCaig AE, Phillips CG, Embley TM, Prosser JI (1998) Analysis of b-subgroup proteobacterial ammonia oxidizer population in soil by denaturing gradient gel electrophoresis analytical and hierarchical phylogenetic probing. *Appl. Environ. Microbiol.* 64:2958-2965