

THE EFFECT OF SOIL CONTAMINATION WITH DIESEL OIL AND PETROL ON THE NITRIFICATION PROCESS

**Jan Kucharski, Monika Tomkiel, Edyta Boros,
Jadwiga Wyszowska**

**Chair of Microbiology
University of Warmia and Mazury in Olsztyn**

Abstract

The effect of soil contamination with diesel oil and petrol on the nitrification process was investigated in a laboratory experiment. Samples of typical brown soil developed from loamy sand, of pH of 6.6 in 1M KCl, Hh $-11.38 \text{ mmol}^+ \text{ kg}^{-1}$ soil, S $- 77.67 \text{ mmol}^+ \text{ kg}^{-1}$ soil and $C_{\text{org}} - 8.50 \text{ g kg}^{-1}$ were analyzed. The experiment was performed in three replications, and for each test 100 g air-dry soil sample was placed in 150 cm³ beakers. Soil samples were contaminated with diesel oil and petrol with the addition of rapeseed oil and ethanol. The source of nitrogen was ammonium sulfate in the amount of 0 and 250 mg N per kg⁻¹ soil. The content of N-NO₃⁻ and N-NH₄⁺ was determined on experimental days 14, 28 and 42. Soil moisture was kept constant at 50% capillary water capacity throughout the experiment.

Fertilizer nitrogen was subject to strong immobilization in soil contaminated with diesel oil and petrol. Both pollutants strongly inhibited the nitrification process. Diesel oil had a much stronger inhibitory effect on nitrification than petrol. Rapeseed oil also proved to be a powerful inhibiting factor. On experimental day 42, diesel oil reduced ammonium cation oxidation by 99%, and petrol – by 88%.

Key words: diesel oil, petrol, soil, nitrification, N-NH₄⁺, N-NO₃⁻.

PROCES NITRYFIKACJI W GLEBIE ZANIECZYSZCZONEJ OLEJEM NAPĘDOWYM I BENZYNĄ

Abstrakt

W doświadczeniu laboratoryjnym badano wpływ zanieczyszczenia gleby olejem napędowym i benzyną na przebieg procesu nitryfikacji. Do badań wykorzystano próbki gleby brunatnej w ściągłej wytworzonej z piasku gliniastego o pH w 1M KCl 6,6, Hh – 11,38 mmol⁺ kg⁻¹ gleby, S – 77,67 mmol⁺ kg⁻¹ gleby, C_{org} – 8,50 g kg⁻¹. Badania wykonano w trzech powtórzeniach, umieszczając w zlewkach o pojemności 150 cm³ po 100 g powietrznie suchej gleby. Próbkę glebową zanieczyszczono olejem napędowym, benzyną i domieszką oleju rzepakowego i etanolu. Jako źródło azotu zastosowano siarczan amonu w ilości 0 i 250 mg N kg⁻¹ gleby. Zawartość N-NO₃⁻ i N-NH₄⁺ oznaczono w 14., 28. i 42. dniu zakończenia doświadczenia. Przez cały okres trwania badań utrzymywano stałą wilgotność gleby na poziomie 50% kapilarnej pojemności wodnej.

Stwierdzono, że w glebie zanieczyszczonej olejem napędowym i benzyną zachodziła silna immobilizacja azotu nawozowego. Obydwa zanieczyszczenia silnie hamowały proces nitryfikacji. Zdecydowanie większe zakłócenia w przebiegu procesu nitryfikacji powodował olej napędowy niż benzyna. Również silnym inhibitorem nitryfikacji okazał się olej rzepakowy. W 42. dniu trwania doświadczenia olej napędowy zmniejszył utlenianie kationu amonowego o 99%, a benzyna o 88%.

Słowa kluczowe: olej napędowy, benzyna, gleba, nitryfikacja, N-NH₄⁺, N-NO₃⁻.

INTRODUCTION

The nitrification process plays a key role in the nitrogen cycle, and nitrifying bacteria are particularly sensitive to environmental conditions. Soil quality can be evaluated in view of the counts of nitrifying bacteria and the intensity of the nitrification process (CASTALDI et al. 2009, DINCER, KARGI 2000, KUCHARSKI 2000, SIMEK 2000). Soil type also affects the process as soils with a high sorptive capacity can inhibit the toxic effect of polluting substances, thus enhancing the intensity of nitrification (MYEKÓW et al. 1996, WYSZKOWSKA, KUCHARSKI 2001).

Increased concentrations of oil-derivative compounds in soil modify the soil's physical and chemical properties, its structure, as well as the composition and populations of soil microbes (KUCHARSKI et al. 2004, WYSZKOWSKA, KUCHARSKI 2001). According to BARABASZ (1992), nitrogen mineralization plays an important role in supplying plants with nutrients, but environmental pollution caused by oil-derivative products which reach the soil as a result of extraction and further processing may lead to adverse changes in the process of nitrogen transformation (AMADI et al. 1996, JORGENSEN et al. 2000).

The supply of foreign substances to the soil environment may alter the soil's biochemical properties. This study attempted to determine the effect of soil contamination with diesel oil and petrol on the content of mineral nitrogen.

MATERIAL AND METHODS

The effect of soil contamination with diesel oil and petrol on the nitrification process was determined in the study. Samples were collected from the humus horizon of soil classified under natural conditions as typical brown soil developed from loamy sand, of pH of 6.6 in 1M KCl, hydrolytic acidity (Hh) of 11.38 mmol⁺ kg⁻¹, total exchangeable alkaline cations (S) of 77.67 mmol⁺ kg⁻¹ and organic carbon content (C_{org}) of 8.50 g kg⁻¹.

The variable experimental factors were:

1. Type of pollutant in cm³·kg⁻¹ d.m. soil:
 - 0 (control),
 - DO (diesel oil – 10 cm³),
 - P (petrol – 10 cm³),
 - R (rapeseed oil – 1 cm³),
 - E (ethanol – 1 cm³),
 - DOR (DO 9 cm³ + R 1 cm³),
 - PE (P 9 cm³ + E 1 cm³).
2. Ammonium sulfate dose in mg N kg⁻¹ soil: 0 and 250.
3. Time of analysis – days 0, 14, 28, 42.

The experiment was conducted in 3 replications. For each test, 100 g of air-dried soil was placed in 150 cm³ beakers. Soil samples were contaminated (variable 1) and fertilized with ammonium sulfate (variable 2). All components were thoroughly mixed with the soil. The moisture content of soil was brought to 50% capillary water capacity. The beakers were incubated at a temperature of 25°C, and N-NH₄⁺ and N-NO₃⁻ levels were determined in the soil on different days of the experiment (variable 3). The detailed procedure of extracting and determining mineral nitrogen is presented by KUCHARSKI et al. (2009). The quantity of nitrified nitrogen and % inhibition of the nitrification process were determined based on the results (WYSZKOWSKA 2002).

The results were processed statistically with the use of Duncan's multiple range test and a three-factorial analysis of variance. A statistical analysis was performed in the Statistica application (StatSoft, Inc. 2006).

RESULTS AND DISCUSSION

The results of the experiment indicate that soil contamination with oil-derivative products modified the nitrification process. Diesel oil and petrol with the addition of rapeseed oil and ethanol had a varied effect on N-NH₄⁺ and N-NO₃⁻ concentrations (Tables 1 and 2).

Table 1

The effect of soil contamination with oil-derivative substances, rapeseed oil and ethanol on N-NH₄⁺ levels (mg kg⁻¹ soil)

Type of pollutant *	Time of analysis (days)			
	0	14	28	42
0 mg N kg ⁻¹ d.m. soil				
0	28.60 ± 0.46	24.63 ± 0.45	22.57 ± 0.59	4.08 ± 0.37
DO	28.83 ± 0.15	23.03 ± 0.23	21.20 ± 1.00	5.52 ± 0.37
P	65.60 ± 0.17	40.90 ± 0.72	21.60 ± 0.70	19.20 ± 1.86
R	28.47 ± 0.23	27.10 ± 0.36	22.23 ± 0.35	11.76 ± 0.37
E	23.83 ± 0.06	23.20 ± 0.44	20.70 ± 0.44	3.24 ± 0.39
DOR	23.97 ± 0.60	22.17 ± 0.55	20.33 ± 0.40	11.28 ± 0.37
PE	64.27 ± 0.25	37.37 ± 0.49	22.57 ± 0.06	15.60 ± 1.86
250 mg N kg ⁻¹ d.m. soil				
0	217.67 ± 0.58	155.33 ± 3.06	30.77 ± 0.81	5.40 ± 0.39
DO	221.00 ± 1.73	211.00 ± 7.21	37.83 ± 0.75	20.64 ± 0.37
P	236.00 ± 2.65	154.33 ± 3.21	103.00 ± 1.73	24.72 ± 2.71
R	218.33 ± 2.89	34.87 ± 0.55	20.90 ± 0.36	4.56 ± 0.37
E	227.33 ± 2.52	190.67 ± 2.08	52.77 ± 0.35	7.56 ± 0.39
DOR	234.67 ± 2.52	34.03 ± 0.32	23.70 ± 0.17	11.40 ± 0.59
PE	236.00 ± 3.61	146.33 ± 3.51	94.60 ± 0.26	23.04 ± 2.28
LSD _{0.01} **	<i>a</i> - 1.29, <i>b</i> - 0.69, <i>c</i> - 0.97, <i>a</i> · <i>b</i> - 1.82, <i>a</i> · <i>c</i> - 2.57, <i>b</i> · <i>c</i> - 1.37, <i>a</i> · <i>b</i> · <i>c</i> - 3.63			

*DO - diesel oil, P - petrol, R - rapeseed oil, E - ethanol

**LSD_{0.01} for: *a* - type of oil-derivative substance and the addition of rapeseed oil and ethanol, *b* - nitrogen source, *c* - time of analysis.

Ammonia nitrogen levels decreased on successive days of the experiment. The highest N-NH₄⁺ concentrations were noted on day 1, and the lowest - on day 42. Petrol contamination had a more profound effect on the content of ammonia nitrogen than diesel oil pollution. In a treatment with petrol without ammonium sulfate, high levels of NH₄⁺ (40.90 mg kg⁻¹) were noted over 14 days, whereas in treatments with ammonium sulfate - over 28 days (103 mg N-NH₄⁺ kg⁻¹ d.m. soil). A reverse tendency was observed in respect of the nitrate nitrogen content of soil. The lowest quantities were determined in soil analyzed on the first day, and the highest - on the last (42nd) day of the experiment. Nitrate nitrogen concentrations were higher in soil contaminated with petrol than with diesel oil throughout the entire experiment.

Table 2

The effect of soil contamination with oil-derivative substances, rapeseed oil and ethanol on N-NO_3^+ levels (mg kg^{-1} soil)

Type of pollutant *	Time of analysis (days)			
	0	14	28	42
0 mg N kg ⁻¹ d.m. soil				
0	7.39 ± 0.03	9.29 ± 0.07	21.53 ± 0.15	26.13 ± 1.07
DO	7.10 ± 0.10	9.11 ± 0.03	0.00 ± 0.00	0.83 ± 0.00
P	7.28 ± 0.06	9.16 ± 0.02	11.37 ± 0.35	24.06 ± 1.07
R	6.61 ± 0.02	8.83 ± 0.08	10.23 ± 0.12	2.13 ± 0.17
E	6.52 ± 0.11	8.36 ± 0.04	9.57 ± 0.04	19.59 ± 1.13
DOR	7.32 ± 0.04	9.27 ± 0.05	0.00 ± 0.00	1.86 ± 0.23
PE	7.32 ± 0.03	8.77 ± 0.04	10.27 ± 0.21	20.28 ± 0.84
250 mg N kg ⁻¹ d.m. soil				
0	8.47 ± 0.37	41.57 ± 0.15	146.00 ± 1.73	240.97 ± 2.03
DO	7.33 ± 0.03	14.87 ± 0.47	0.00 ± 0.00	3.03 ± 0.21
P	7.54 ± 0.26	18.33 ± 0.21	47.53 ± 0.32	29.91 ± 1.13
R	6.83 ± 0.15	14.37 ± 0.15	43.47 ± 0.06	89.72 ± 1.13
E	6.67 ± 0.09	26.27 ± 0.15	125.67 ± 4.93	212.44 ± 1.84
DOR	7.96 ± 0.05	11.53 ± 0.32	0.00 ± 0.00	2.27 ± 0.23
PE	7.58 ± 0.23	17.43 ± 0.31	34.73 ± 0.45	24.06 ± 1.07
LSD _{0.01} **	$a - 0.71, b - 0.38, c - 0.53, a \cdot b - 0.99, a \cdot c - 1.41, b \cdot c - 0.75, a \cdot b \cdot c - 1.99$			

*DO – diesel oil, P – petrol, R – rapeseed oil, E – ethanol

**LSD_{0.01} for: a – type of oil-derivative substance and the addition of rapeseed oil and ethanol, b – nitrogen source, c – time of analysis.

Soil contamination with diesel oil and petrol had an adverse effect on nitrified ammonium sulfate levels (Table 3). The addition of rapeseed oil and ethanol contributed to the above trend. Diesel oil administered separately and with the addition of rapeseed oil proved to be a stronger inhibitor. In these treatments, negligent quantities of nitrate nitrogen were noted on day 42.

Diesel oil and petrol strongly inhibited the nitrification process (Table 4). Diesel oil had a much stronger inhibitory effect on nitrification than petrol. The addition of rapeseed oil as well as ethanol strongly contributed to the adverse effect of oil derivatives on the nitrification process. On day 42, diesel oil reduced cation oxidation by 99%, and petrol – by 88% in treatments with ammonium sulfate. The tested substances inhibited nitrification in the following order: diesel oil > diesel oil + rapeseed oil > rapeseed oil > petrol + ethanol > petrol > ethanol.

Table 3

The effect of soil contamination with oil-derivative substances, rapeseed oil and ethanol on the levels of nitrified nitrogen in soil fertilized with ammonium sulfate (%)

Type of pollutant *	Time of analysis (days)		
	14	28	42
0	12.91 ± 0.08	49.79 ± 0.75	85.94 ± 0.81
DO	2.30 ± 0.20	0.00 ± 0.00	0.88 ± 0.09
P	3.67 ± 0.08	14.47 ± 0.10	2.34 ± 0.62
R	2.21 ± 0.08	13.29 ± 0.02	35.04 ± 0.49
E	7.16 ± 0.07	46.44 ± 1.98	77.14 ± 0.87
DOR	0.91 ± 0.11	0.00 ± 0.00	0.17 ± 0.00
PE	3.47 ± 0.11	9.79 ± 0.10	1.51 ± 0.62
LSD _{0.01} **	$a - 0.56, b - 0.42, a \cdot b - 1.12$		

*DO – diesel oil, P – petrol, R – rapeseed oil, E – ethanol

**LSD_{0.01} for: a – type of oil-derivative substance and the addition of rapeseed oil and ethanol, b – nitrogen source, c – time of analysis.

The results of the study indicate that diesel oil was a more powerful inhibitor of the nitrification process than petrol. The adverse effect of oil-derivative substances on nitrification was noted by KUCHARSKI et al. (2004). The above authors found that leaded and unleaded petrol administered in the amount of 6 cm³ kg⁻¹ d.m. soil had a stronger inhibitory effect on nitrification than diesel oil. An opposite effect was noted in this study, most probably due to higher doses (10 cm³ kg⁻¹ d.m. soil) of the tested oil-derivative substances than those applied in the above cited experiment.

The studies carried out by AMADI et al. (1996), NIEWOLAK and KOZIEŁŁO (1998) validated the negative effect of oil-derivative substances on the nitrification process. The above authors observed that nitrifying bacteria were highly sensitive to soil contamination with crude oil. The adverse effect of pollution with oil-derivative substances on the soil's biological activity was also noted by XU et al. (2000). The results of the above study pointed to the high sensitivity of bacteria of the genera *Nitrosomonas* and *Nitrobacter* to the tested substances.

KUCHARSKI et al. (2004), NIEWOLAK and KOZIEŁŁO (1998) argued that the dynamics of the nitrification process increases over time as hydrocarbon levels decrease due to evaporation and mineralization. Another crucial factor is the selection of microbes resistant to the toxic effect of soil pollutants. The findings of SCHIE and YOUNG (1998) indicate that low-biodegradable fuel compounds may be metabolized by microorganisms.

Table 4

The nitrification-inhibiting effect of oil-derivative substances,
rapeseed oil and ethanol (%)

Type of pollutant *	Time of analysis (days)		
	14	28	42
0 mg N kg ⁻¹ d.m. soil			
0	1.90 ± 0.42	100.00 ± 0.00	96.84 ± 0.13
DO	1.40 ± 0.56	47.21 ± 1.77	7.91 ± 0.33
P	4.95 ± 0.57	52.48 ± 0.36	91.84 ± 0.61
R	10.04 ± 0.56	55.54 ± 0.50	24.89 ± 5.35
E	0.21 ± 0.18	100.00 ± 0.00	92.68 ± 0.73
DOR	5.63 ± 0.23	52.32 ± 1.01	22.22 ± 5.23
PE			
0	64.23 ± 1.25	100.00 ± 0.00	98.74 ± 0.10
DO	55.89 ± 0.38	67.44 ± 0.30	87.59 ± 0.37
P	65.44 ± 0.37	70.23 ± 0.38	62.76 ± 0.61
R	36.81 ± 0.23	13.90 ± 4.06	11.83 ± 1.18
E	72.25 ± 0.78	100.00 ± 0.00	99.06 ± 0.09
DOR	58.06 ± 0.82	76.21 ± 0.40	90.02 ± 0.39
PE	$a - 1.68, b - 0.97, c - 1.37, a \cdot b - 2.37, a \cdot c - 3.36, b \cdot c - 1.94, a \cdot b \cdot c - 4.75$		

*DO – diesel oil, P – petrol, R – rapeseed oil, E – ethanol

**LSD_{0.01} for: a – type of oil-derivative substance and the addition of rapeseed oil and ethanol, b – nitrogen source, c – time of analysis.

CONCLUSIONS

1. Soil contamination with diesel oil and petrol has a negative effect on the nitrification process.
2. Diesel oil is a stronger inhibitor of the nitrification process than petrol.
3. The addition of rapeseed oil to diesel oil and the addition of ethanol to petrol reinforces the adverse effect of the studied oil-derivative substances on the dynamics of ammonium nitrogen nitrification in soil.

REFERENCES

AMADI A., ABBEY S.D., NMA A. 1996. *Chronic effects of oil spil on soil properties and microflora of rainforest ecosystem in Nigeria*. Water, Air Soil Pollut., 86: 1-11.

- BARABASZ W. 1992. *Mikrobiologiczne przemiany azotu glebowego [Microbiological transformations of soil nitrogen]*. Post. Mikrobiol., 31(1): 3-29 (in Polish).
- CASTALDI S., CARFORA A., FIORENTINO A., NATALE A., MESSERE A., MIGLIETTA F., COTRUFO M.F. 2009. *Inhibition of net nitrification activity in a Mediterranean woodland: possible role of chemicals produced by *Arbutus unedo**. Plant Soil, 315: 273-283.
- DINCER A.R., KARGI F. 2000. *Kinetics of sequential nitrification and denitrification processes*. Enz. Microbiol. Technol., 27: 37-42.
- JØRGENSEN K.S., PUUSTINEN J., SUORTI A.M. 2000. *Bioremediation of petroleum hydrocarbon-contaminated soil by composting in biopiles*. Environ. Pollut., 107: 245-254.
- KUCHARSKI J., WYRWAŁ A., BOROS E., WYSZKOWSKA J. 2009. *The nitrification process as an indicator of soil contamination with heavy metals*. Ecol. Chem. Eng. (in press)
- KUCHARSKI J., JASTRZĘBSKA E., WYSZKOWSKA J. 2004. *Effects of some oil products on the course of ammonification and nitrification*. Acta Agr. Silv. ser. Agr., 42: 249-255 (in Polish)
- KUCHARSKI J. 2000. *The importance of nitrification*. In: *Microbiology at the turn of the centuries*. Ed. A. SIWIK, 37-40 (in Polish).
- MYĘKÓW W., STACHYRA A., ZIĘBA S., MASIĄK D. 1996. *Biological activity of soil as an indicator of its fertility*. Roczn. Glebozn., 37(1/2): 89-99.
- NIEWOLAKA S., KOZIEŁŁO M. 1998. *Intensity of some nitrogen transformations in soils experimentally contaminated with crude oil*. Pol. J. Environ. Stud., 7(3): 161-168.
- SCHIE P.M., YOUNG L.Y. 1998. *Isolation and characterization of phenol-degrading denitrifying bacteria*. Appl. Environ. Microbiol., 64(7): 2432-2438.
- SIMEK M. 2000. *Nitrification in soil – terminology and methodology*. Rostl. Vyr., 46 (9): 385-395.
- StatSoft, Inc. 2006. *Statistica (data analysis software system), version 6.0*. www.statsoft.com.
- WYSZKOWSKA J. 2002. *Biological properties of soil contaminated with hexavalent chromium*. Wyd. UWM, Rozpr. i Monogr., 65: 1-134 (in Polish).
- WYSZKOWSKA J., KUCHARSKI J. 2004. *Process of nitrification in soil contaminated with fuel oil*. Roczn. Glebozn., 55(2): 517-525 (in Polish).
- WYSZKOWSKA J., KUCHARSKI J. 2001. *Role of *Azotobacter* sp. and adenine in yield formation of lettuce and bacteria abundance in soil*. Scientific works of the Lithuanian Institute of Horticulture and Lithuanian University of Agriculture, 20(3)-2: 279-285.
- WYSZKOWSKA J., KUCHARSKI J. 2001. *Correlation between number of microbes and degree of soil contamination by petrol*. Pol. J. Environ. Stud., 10(3): 175-181.
- XU H., CHEN G., LI A., HAN S., HUANG G., XU H., CHEN G., LI A., HUANG G. 2000. *Nitrification and denitrification as sources of gaseous nitrogen emission from different forest soils in Changbai mountain*. J. Foresty Res., 11(3): 177-182.