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NITRIFICATION POTENTIAL AS INDICATOR OF PAHs ECOTOXICITY IN FRESHLY CONTAMINATED SOILS. EXAMPLE OF PHENANTHRENE AND PYRENE

POTENCJAŁ NITRYFIKACJI JAKO WSKAŹNIK EKOTOKSYCZNOŚCI WWA W GLEBACH ŚWIEŻO ZANIECZYSZCZONYCH. NA PRZYKŁADZIE FENANTRENU I PIRENU

Abstract: The objective of the study was to evaluate the possibility of application of nitrification potential as an indicator of the ecotoxicity of PAHs to soil microorganisms in freshly contaminated soils. The effects of two model PAHs compounds phenanthrene and pyrene were studied under laboratory conditions (incubation of soils for 7 days at 20 ± 2 °C). Eight soil materials originated from ploughing layer (0–20 cm) of soils not exposed to direct PAH sources were applied. Soil materials were spiked with phenanthrene or pyrene at the levels of 1, 10, 100 and 500 mg · kg^{-1}. Contamination of soils with PAHs inhibited the activity of nitrifying bacteria, which appeared to be a sensitive indicator of the presence of PAHs. The effect of hydrocarbons was related to the soil characteristic and compound properties; the strongest inhibition corresponded to light soils with low organic matter content and low biological activity contaminated with phenanthrene, characterized by high water solubility and bioavailability. The high acidity of soils created additional stress to nitrifying bacteria and thus increased their susceptibility to the effect of contaminants such as PAHs.

Keywords: nitrification potential, polycyclic aromatic hydrocarbons, phenanthrene, pyrene, ecotoxicity parameters

There is an increasing interest in developing indicators for evaluation of soil quality, to protect effectively soil habitat function. Soils from agricultural land are often exposed to the influence of stressors such as chemical contaminants. Although parameters describing general microbial activity (eg respiration, total biomass) are not considered to be the good indicators of soil pollution, there are some specific groups of soil microorganisms exhibiting high sensitivity to organic contaminants like PAHs [1–3].

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The good example are nitrifying bacteria playing important role in the cycling of nitrogen in soil environment. They are very sensitive to low concentration of contaminants and rapidly response to soil perturbation [4–6].

Polycyclic aromatic hydrocarbons (PAHs) represent the group of persistent organic pollutants (POPs). Some of them are resistant to physical, chemical and biological degradation and can remain in the environment for a long time [3, 7]. PAHs originate from incomplete combustion or pyrolysis of organic substances like coal, oil, petrol, etc. The other sources of PAHs to soil are disposal of waste materials, creosote use and road runoffs, accidental fuel spills and leakages as well as industrial wastewaters, sewage sludge and compost applied to agricultural land [8, 9]. PAHs incorporated into soil may undergo several processes. The main mechanisms include biodegradation, chemical transformation, volatilization, photolysis, sorption to the soil solid phase, leaching and transfer to plants and grazing animals [3, 8, 10]. PAHs compounds are generally hydrophobic and non-volatile, but individual hydrocarbons exhibit different physicochemical properties, which control their fate, reactivity and effects on soil ecosystems [10, 11].

The main objective of this study was to evaluate the possibility of application of nitrification potential as an indicator of the ecotoxicity of PAHs to soil microorganisms in freshly contaminated soils. Phenanthrene (Phen) and pyrene (Pyr) – the compounds exhibiting high ecotoxic activity [1, 12–14] and being abundant in the soil environment [9] were chosen as model PAHs. The particular attention was paid to soil properties regulating nitrifying bacteria activity and PAHs bioavailability [6, 10, 15].

Materials and methods

Soils characteristic

Eight different soils were used in the laboratory experiments. All soil samples were collected from the typical rural areas (Lublin province, Poland) not exposed to direct PAH sources, from 0–20 cm layer. After transport to the laboratory, soil material was air dried at 20 ^{o}C, well mixed, sieved to pass a 2 mm sieve-mesh and stored for no longer than 6 months in the dark (12–16 ^{o}C) before soil physicochemical characteristic and ecotoxicity testing.

Soil characteristics included the determination of particle size distribution, soil organic carbon content, pH and content of PAHs. Soil particle size distribution was established by an aerometric method [16]. Soil organic carbon (C_{org}) content was determined by sulfochromic oxidation of organic carbon followed by titration of the excess $K_2Cr_2O_7$ with $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$ [17]. The pH was measured potentiometrically in 1:2.5 (m/V) suspension of soil in 1 mol $\cdot$ dm^{-3} KCl solution [18].

Thirteen PAH (Σ13PAH) compounds (US EPA list) were determined by extraction with dichloromethane in Soxtec apparatus (Büchi Universal Extraction System B-811) for 6 h. The extracts were concentrated to a volume of 1 cm^3 under vacuum on a rotary evaporator and cleaned up on glass minicolumns (0.5 × 20 cm) filled with 1 g of silica gel (conditioned at 135 ^{o}C for 16 h) suspended in dichloromethane. PAHs were eluted with 5 cm^3 of a mixture of CH_2Cl_2/*n*-heksane (2:3 v/v). The eluate was evaporated to

a volume of approximately 1 cm^3 and analyzed on GCQ MAT Finigan gas chromatograph equipped with MS detector with ion trap. Resolution of PAH compounds has been achieved with DB-5 MS fused-silica capillary column 30 m × 0.25 mm I.D. with a film thickness of 0.25 μm and with 10 m guarded column (J&W Scientific, USA). Helium was used as a carrier gas (constant flow of 40 $cm \cdot s^{-1}$) with a splitless injection system at 250 ^oC. The GC oven was programmed as follows: 35 ^oC for 2 min, followed by a 30 $^oC\ min^{-1}$ ramp to 120 ^oC and then with ramp of 5 $^oC\ min^{-1}$ to final temperature of 290 ^oC (10 min hold). Mass spectrometer (MS) detection was based on selected ion monitoring (SIM) system. The solvent blank sample was carried out through all procedures. Quality control included analysis of a reference soil sample (soil No. 701 from SETOC program, 1992–1995) every 20 samples. The precision of the method corresponding to the mean relative standard deviation (RSD) was in the range of 2 to 24 % for individual PAH compounds and 8 % for the sum of 13 PAH compounds. The mean recovery calculated for 13 PAHs in the reference soil was 71 %, with recovery for individual compounds ranged from 53 % for benzo[b]fluoranthene to 112 % for phenanthrene.

PAH characteristic

Two PAH compounds, differing substantially with their physicochemical properties, were used in the studies. 3-ring phenanthrene (M_w = 178) has high water solubility = = 1300 $\mu g \cdot dm^{-3}$, log K_{ow} = 4.57 and Henry's constant = 3.24 $Pa \cdot m^3 \cdot mol^{-1}$, while 4-ring pyrene ($M_w$ = 202) has water solubility = 132 $\mu g \cdot dm^{-3}$, log K_{ow} = 5.18 and and Henry's constant = 0.92 $Pa \cdot m^3 \cdot mol^{-1}$ [11]. Water/organic carbon partition coefficients (K_{oc}), describing sorption affinity of PAHs to soil organic matter, corresponded to 19055 $dm^3 \cdot kg^{-1}$ and 45709 $dm^3 \cdot kg^{-1}$ for phenanthrene and pyrene, respectively [19].

The stock solutions of hydrocarbons were prepared by dissolving 25 g of Phen and Pyr in 1000 cm^3 dichloromethane, stored in the dark at room temperature and diluted further with CH_2Cl_2 according to needs.

Four levels of two PAHs were applied:

I) 1 $mg \cdot kg^{-1}$, corresponding to Σ9PAHs threshold value for agricultural soils according Polish [20] and Dutch regulations [21] and corresponding to Danish ecotoxicological criterion for PAHs in soil [22];

II) 10 $mg \cdot kg^{-1}$, corresponding to German guideline value for soil with respect to the growth and quality of plants [23, 24];

III) 100 $mg \cdot kg^{-1}$, corresponding to German guideline value for soil in industrial areas [24];

IV) 500 $mg \cdot kg^{-1}$, corresponding to PAHs action level according the UK regulations [23].

Experimental procedure

The soils subsamples (100 ± 0.1 g) were placed in glass beakers and spiked with 2 cm^3 of dichloromethane solution of phenanthrene and pyrene at the levels of: 1, 10, 100

and 500 mg of each hydrocarbon *per* kg of dry soil. Each soil sample, after careful mixing with appropriate amount of Phen-CH_2Cl_2 or Pyr-CH_2Cl_2 solution, was left overnight to let the solvent evaporate. After 24 hours the subsamples were supplemented with 200 ± 0.1 g of soil, thoroughly mixed, moistened with deionized water to 55 % water holding capacity and incubated in the dark for 7 days at 20 ± 2 °C. Soil moisture content was kept at the constant level by periodically weighing the samples and adding water as necessary. Non-spiked soil samples amended with pure dichloromethane (at the amount corresponding to those used in PAH-spiked soils) were applied as a control (zero treatment). Each treatment was replicated two times. After 7 days the soils were mixed and two subsamples (25 ± 0.1 g wet weight) were taken from the each replicate for nitrification potential determinations.

Determination of soil nitrifying bacteria activity

Nitrification potential in soil was determined according to ISO 15685 [25] method. Moist soil subsamples (25 ± 0.1 g) were placed in a 250 cm^3 glass flask and mixed with the mineral medium to form slurry. The volume of medium was calculated by subtracting the volume of water in the initial soil sample from the desired liquid volume 100 cm^3. The mineral medium contained 1.5 mmol · dm^{-3} of diammonium sulphate $(NH_4)_2SO_4$ as a substrate, 1 mmol · dm^{-3} of potassium phosphate buffer (KH_2PO_4 and K_2HPO_4) and 5.625 mmol · dm^{-3} of sodium chlorate(V) (to prevent further oxidation of NO_2^- to NO_3^-). The pH of the medium was approximately 7.2. The soil slurries were mixed for 6 hours on a shaker at approximately 175 rpm at room temperature 20 ± 2 °C. After incubation 2 cm^3 of the slurry were placed in 25-cm^3 glass beakers and 2 cm^3 of 4 mol · dm^{-3} KCl were added to stop the ammonium oxidation. The suspension was filtered using 390-grade filter paper. Then, 1 cm^3 of the filtrate was transferred to glass flask filled with 20 cm^3 of deionised water and 0.2 cm^3 of the colour reagent containing sulphanilamide ($C_6H_8N_2O_2S$) and *N*-(1-naphtyl)ethylene diamine dihydrochloride ($C_{12}H_{16}N_2Cl_2$). After 60 min, the intensity of the purple colour was measured on Beckman DU-68 spectrophotometer at λ = 543 nm. In each series of measurements the control samples without soil material were applied. All determinations were done in duplicates for each of the replicate soil samples and the results for individual samples were expressed as an arithmetic mean (μg NO_2^- · g^{-1}) of two measurements adjusted to soil dry matter (105 °C). The final results were given as an arithmetic mean of four measurements (2 replicates × 2 NP determinations). For ANOVA evaluations, the mean values for the each of the replicates (n = 2) were used.

Precision of the method (corresponding to the RSD values for n = 10) was about 5–8 % for one soil sample and 5–15 % for the replicates of 10 samples (n = 20).

Statistics

To enable comparison of the data for different soils, the results of NP determinations were related to the control (100 %) and expressed as relative nitrification potential (RNP). The analysis of variance method (one-way ANOVA, Tukey HSD test) was

applied for the statistical evaluation of the effects. Before ANOVA was performed, the variance check (Bartlett's test appropriate for equal and unequal group size) was done to examine if the samples were from the same populations. Pearson product moment correlations were used for evaluation of relationship between each pair of variables. Normality of all data was checked using standardized skewness and standardized kurtosis parameters.

The ecotoxicity parameters were evaluated on the basis of the determination of the effects of these contaminants on soil microorganisms. The ecotoxic effects of PAHs were expressed as NOEC (*No Observed Effect Concentration*), LOEC (*Lowest Observed Effect Concentration*), EC_{20} and EC_{50} (concentration of Phen or Pyr in soil causing 20 % and 50 % inhibition of NP, respectively). LOEC is the lowest tested concentration that results in a statistically significant adverse effect (in relation to the control). The NOEC is defined as the highest test concentration below the LOEC. EC_{20} and EC_{50} values were calculated on the basis of the best-fit simple regression models (concentration – effect relationship). Statistical evaluations were done using Statgraphics Centurion version XV program.

Results

The applied soils differed in their characteristics, although the range of their properties was not very wide – Table 1. Three soils (No 3–No 5) represented sands, three another (No 6–No 8) – loams, and two soils (No 11–No 12) represented silts. As regards physicochemical properties, the highest variability (CV of 63–77 %) corresponded to the fraction < 0.002 mm and C_{org} contents, while biological activity, expressed by the NP values, differed over one order of magnitude (0.26–5.76 μg $NO_2^- \cdot g^{-1}$). The content of Σ13PAHs in soils was consistently low, much below the limit values set by Polish regulations for the top layer of agricultural soils; the exception was soil No 11, where the concentration of phenanthrene exceeded the limit value of 100 μg · kg^{-1} [20]. Generally, the soils exhibited properties typical for Polish agricultural land; they were slightly acidic, with low organic matter content and low level of contamination with PAHs [9, 26].

Amendments of the soils with Phen at the lowest applied level (1 mg · kg^{-1}) caused statistically significant inhibition of nitrification potential in four soils (No 3, No 6, No 8 and No 12 – Table 2) with the strongest effect in soil No 3 (RNP of 63 %). The effect increased at the next Phen dose (10 mg · kg^{-1}). At the level of 500 mg Phen · kg^{-1} nitrification potential was significantly inhibited in all eight soils, however, high differences between the soils were visible; while RNP in soil No 8 decreased to 75 %, in soil No 11 it was as low as 4 %.

Application of pyrene at the level of 1 mg · kg^{-1} inhibited NP in 6 from 8 tested soils (RNP of 44–91 %) with the exception of soils No 8 and No 12, where the first statistically significant effects were observed at the level 10 and 100 mg · kg^{-1}, respectively (Table 2). Increase of the pyrene level to 500 mg · kg^{-1} caused further decrease of microbial activity (in soil No 3 the nitrification potential was totally inhibited).

Table 1

Basic properties of soil materials (n = 8)

Properties / Texture	Soil code								Statistical parameters		
	No 3	No 4	No 5	No 6	No 7	No 8	No 11	No 12	Mean	SD	CV
	Light loamy sand	Light loamy sand	Heavy loamy sand	Sandy loam	Clay loam	Medium loam	Silty loam	Silty clay loam			
fr. < 0.02	15	14	20	29	33	46	35	49	30	13	44
fr. < 0.002	5	3	8	13	14	32	6	15	12	9	77
C_{org}	7.2	6.3	7.0	16.8	15.4	15.3	9.2	32.1	13.7	8.6	63
pH_{KCl}	5.5	5.4	6.4	6.6	7.0	6.9	5.8	7.0	6.3	0.7	11
C:N	7.2	9.0	10.0	14.2	15.4	13.4	8.4	13.4	11.4	3.1	27
NP_{init}	0.39	0.94	0.80	5.76	2.99	4.27	0.26	3.37	2.35	2.05	87
$Phen_{init}$	28	46	15	48	75	43	144	34	54	40	74
Pyr_{init}	20	23	48	22	36	15	16	21	25	11	45
Σ13PAH	303	427	452	477	460	274	377	378	393	74	19

Texture – classification of textural groups according to Polish standard PN-R-04033; fraction content < 0.02 and < 0.002 mm [%]; C_{org} – organic carbon content [$g \cdot kg^{-1}$]; NP_{init} – nitrification potential at the beginning of the experiment [$\mu g\ NO_2^- \cdot g^{-1}$]; $Phen_{init}$ – initial content of phenathrene [$\mu g \cdot kg^{-1}$]; Pyr_{init} – initial content of pyrene [$\mu g \cdot kg^{-1}$]; Σ13PAH – content of 13 PAHs according to US EPA list [$\mu g \cdot kg^{-1}$]; CV – coefficient of variation [%].

Table 2

The results of nitrification potential (NP) determinations in soils contaminated with phenanthrene and pyrene (range 0–500 mg · kg^{-1}) after 7 days of incubation. The values are means for two replications (n = 2) ± SD

PAH level[1]	NP* [μg NO_2^- · g^{-1}]							
	No 3	No 4	No 5	No 6	No 7	No 8	No 11	No 12
Phenanthrene								
Control 0	0.43 ± 0.04^a	0.99 ± 0.04^a	0.67 ± 0.02^a	4.62 ± 0.21^a	2.66 ± 0.16^a	3.04 ± 0.15^a	0.13 ± 0.01^a	1.90 ± 0.07^a
1	0.27 ± 0.02^b	0.98 ± 0.05^a	0.66 ± 0.05^a	4.17 ± 0.16^b	2.70 ± 0.08^a	2.80 ± 0.08^b	0.13 ± 0.01^a	1.73 ± 0.07^b
10	0.25 ± 0.03^b	0.97 ± 0.04^a	0.52 ± 0.02^b	3.90 ± 0.20^b	2.54 ± 0.08^a	2.72 ± 0.12^b	0.04 ± 0.01^b	1.70 ± 0.04^b
100	0.09 ± 0.02^c	0.73 ± 0.04^b	0.24 ± 0.02^c	3.01 ± 0.17^c	1.99 ± 0.08^b	2.70 ± 0.15^b	0.02 ± 0.00^c	1.42 ± 0.08^c
500	0.04 ± 0.00^c	0.61 ± 0.02^c	0.15 ± 0.02^c	2.63 ± 0.17^c	1.11 ± 0.07^c	2.27 ± 0.20^c	0.01 ± 0.00^d	1.08 ± 0.05^d
Pyrene								
Control 0	0.15 ± 0.02^a	0.87 ± 0.05^a	0.63 ± 0.02^a	3.44 ± 0.20^a	1.96 ± 0.02^a	2.92 ± 0.01^a	0.09 ± 0.02^a	1.72 ± 0.08^a
1	0.10 ± 0.02^b	0.74 ± 0.02^b	0.53 ± 0.02^b	3.12 ± 0.07^b	2.32 ± 0.02^b	2.84 ± 0.07^a	0.04 ± 0.01^b	1.70 ± 0.14^a
10	0.09 ± 0.02^b	0.59 ± 0.02^c	0.58 ± 0.01^c	3.25 ± 0.25^b	2.36 ± 0.03^b	2.72 ± 0.05^b	0.04 ± 0.01^b	1.66 ± 0.02^a
100	0.04 ± 0.01^c	0.48 ± 0.02^d	0.53 ± 0.02^b	2.83 ± 0.08^c	1.96 ± 0.02^a	2.57 ± 0.05^c	0.02 ± 0.00^c	1.60 ± 0.02^b
500	0.00 ± 0.00^d	0.40 ± 0.02^e	0.54 ± 0.08^b	2.76 ± 0.08^c	1.96 ± 0.02^a	1.81 ± 0.07^d	0.01 ± 0.00^d	1.52 ± 0.02^c

[1] Level of PAH [mg · kg^{-1}]; * NP – nitrification potential. The values in columns followed by the same letter are not significantly different (one-way ANOVA, Tukey HSD test) at $p \leq 0.05$ level.

In 15 % of soils the lower levels of contamination (1 and 10 mg · kg^{-1}) led to stimulation of NP activity. Stimulatory effects were most distinct in soil No 7 contaminated with pyrene (Table 2).

Toxic activity of PAHs on nitrification potential was related to PAH properties. The comparison of the reaction of nitrifying bacteria in soils contaminated with phenanthrene and pyrene at the level of 100 and 500 mg · kg^{-1} is presented in Fig. 1. In most of the cases (60 % of soils), stronger effects were observed for Phen than for Pyr.

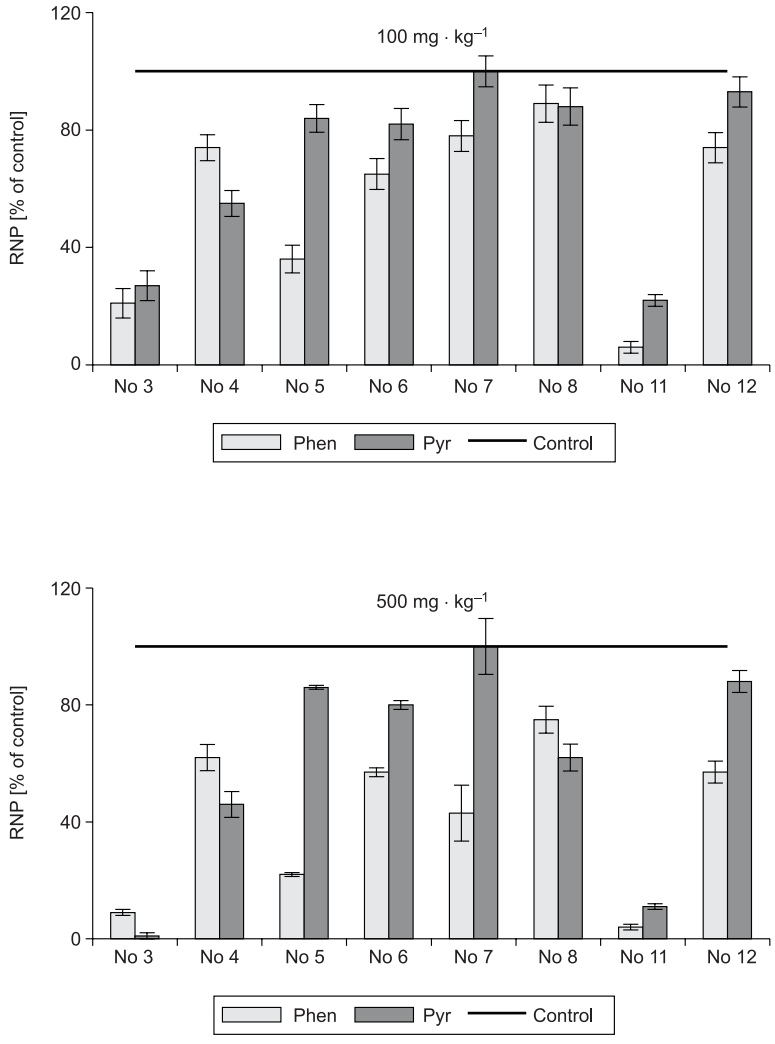

Fig. 1. Comparison of the relative nitrification potential RNP (expressed as a percent of control; control = 100 %) in soils contaminated with phenanthrene and pyrene at the levels of 100 and 500 mg · kg^{-1}. Bars represent 95 % Tukey HSD intervals

Property of soil was the other factor affecting reaction of nitrification bacteria to contamination with PAHs – Table 2. Nevertheless, the correlation coefficients (r) between soil parameters and the effects of PAHs on NP were rather low and significant mainly for pyrene – Table 3. The highest r values for Pyr corresponded to soil acidity (for pH_{KCl} r = 0.63), to C:N ratio (r = 0.65) and to initial nitrification activity of soils (r = 0.49). The correlation between PAHs effect and soil properties was more distinct at the higher doses of PAHs – 100 and 500 mg · kg^{-1}. For further evaluation of those effects the soils were divided into two groups. The first one (soils No 3, No 4, No 5 and No 11) represented low C_{org} contents (< 9.2 g · kg^{-1}), pH values < 6.4 and low microbial activity (NP < 0.94 μg NO_2^- · g^{-1}). The second group of soils (No 6, No 7, No 8 and No 12) had organic carbon content in the limits of 15.3–32.1 g · kg^{-1}, neutral pH (6.6–7.0) and higher values of NP (2.99–5.76 μg NO_2^- · g^{-1}). The significant differences in reaction of microorganisms in both groups of soils were visible at the levels > 100 mg · kg^{-1} (Fig. 2). Both phenanthrene and pyrene exhibited stronger toxic activity in light soils from group I, where RNP values were about 30 % lower than in the group II.

Table 3

Correlation coefficients (r) between soil properties and the effect (NP in % of control) of PAHs on nitrification potential

Soil property	All levels (n = 40)		Phen + Pyr (n = 16)			
	Phen	Pyr	1 mg · kg^{-1}	10 mg · kg^{-1}	100 mg · kg^{-1}	500 mg · kg^{-1}
fr. < 0.002 mm	0.29	0.38*	0.34	0.44	0.60*	0.47*
fr. < 0.02 mm	0.18	0.29*	0.27	0.23	0.42	0.36
C_{org}	0.23	0.41*	0.32	0.42	0.53*	0.48*
pH_{KCl}	0.27	0.63*	0.56*	0.66*	0.72*	0.64*
C:N	0.37*	0.65*	0.64*	0.75*	0.79*	0.72*
NP_{init}	0.34*	0.49*	0.41	0.59*	0.68*	0.62*
Σ13PAH	0.10	0.31*	0.36	0.33	0.21	0.32

Explanations as in Table 1; * statistically significant at the level of $p \leq 0.05$.

To calculate the EC_x parameters, the effect-concentration relationships were evaluated following OECD guidelines [27]. Different linear regression models were tested for EC_{20} and EC_{50} calculations and on the basis of the r^2 values the square root-X regression was chosen as the best-fitting model giving the average r^2 of 85 % for Phen and 76 % for Pyr. The EC_{20} and EC_{50} data (with their 95 % confidence intervals) are given in Table 4; the EC_{50} parameters varied from 47 to 374 mg · kg^{-1} and from 28 to 279 mg · kg^{-1} for soils contaminated with Phen and Pyr, respectively (Table 4). For half of the soils, the effect of hydrocarbons was low, the predicted EC_{50} parameters exceeded the application limit of 500 mg · kg^{-1}. The EC_{20} parameters were within the limits of 14–296 mg · kg^{-1} for phenanthrene and 8–379 mg · kg^{-1} for pyrene.

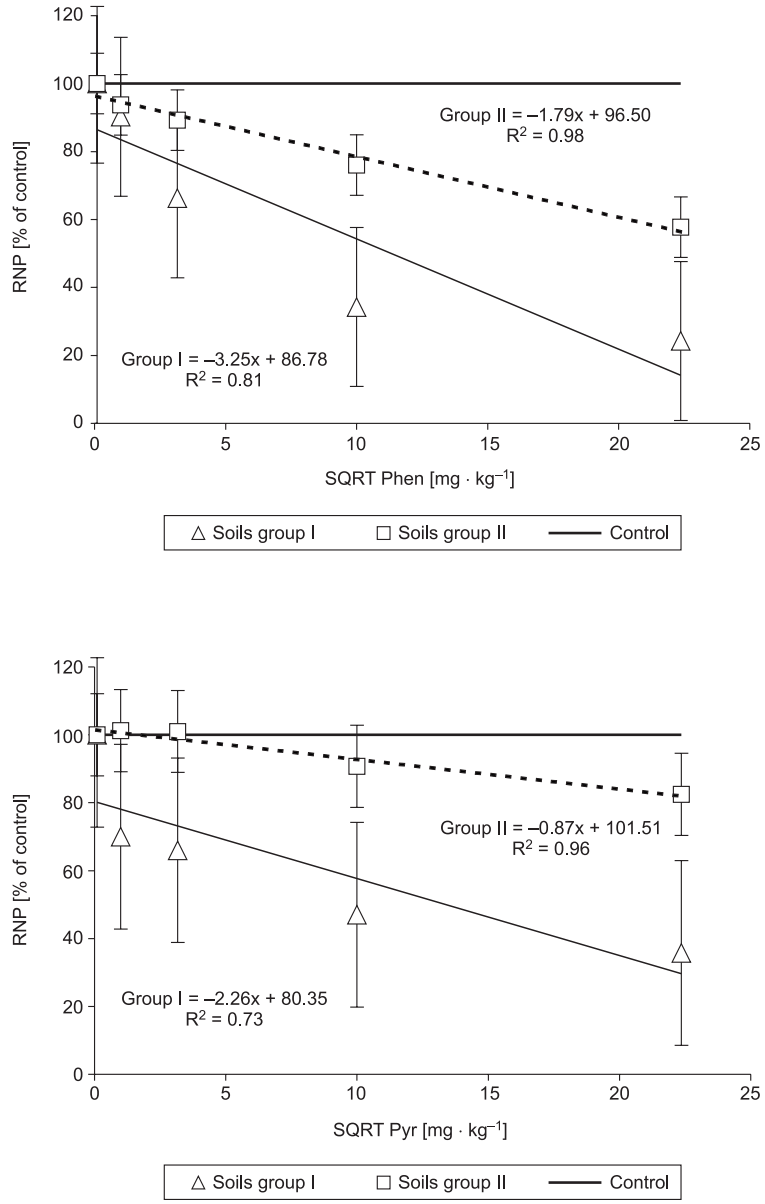

Fig. 2. Mean effects of Phen and Pyr on nitrification potential in two group of soils; group I – soils No 3, No 4, No 5, No 11; group II – soils No 6, No 7, No 8, No 12

In 60 % of evaluated combinations (8 soils × 2 PAHs) the LOEC values corresponded to the lowest applied concentration of 1 mg · kg^{-1}, while in the other cases it reached even 100 mg · kg^{-1}. Consequently, in majority of the samples the evaluated NOEC value was below 1 mg · kg^{-1} (Table 4).

Table 4

PAHs ecotoxicity parameters [mg · kg^{-1}] for soils under study

Parameter	No 3	No 4	No 5	No 6	No 7	No 8	No 11	No 12
Phenanthrene [mg · kg^{-1}]								
EC_{20}[a]	< 1	119 (78–182)	14 (3–38)	48 (21–103)	69 (57–83)	296 (181–498)	< 1	76 (54–105)
EC_{50}[a]	55 (19–144)	HAL	149 (86–260)	HAL	374 (330–425)	HAL	47 (9–192)	HAL
LOEC[b]	1	100	10	1	100	1	10	1
NOEC[b]	< 1	10	1	< 1	10	< 1	1	< 1
Pyrene [mg · kg^{-1}]								
EC_{20}[a]	< 1	8 (0–37)	287 (117–772)	379 (185–840)	NE	152 (121–192)	< 1	HAL
EC_{50}[a]	56 (26–115)	279 (152–534)	HAL	HAL	NE	HAL	28 (2–154)	HAL
LOEC[b]	1	1	1	1	NE	10	1	100
NOEC[b]	< 1	< 1	< 1	< 1	NE	1	< 1	10

[a] 20 and 50 % effect concentration evaluated on the basis of the best-fit square-root-x regression, in brackets – 95 % confidence interval; [b] evaluated on the basis of the results of ANOVA (one-way, Tukey HSD test, at $p \leq 0.05$ level); NE – could not be estimated; HAL – extrapolated values above highest applied level.

Discussion

Presented results indicate that nitrification potential is a very sensitive parameter for description of the ecotoxicity of PAHs in freshly contaminated soils; the lowest observed effect corresponded to 1 mg · kg^{-1}. This is in agreement with opinion, that nitrifying bacteria are the most sensitive group of soil microorganisms exhibiting quick reaction to soil pollution [4, 6]. The negative impact of PAHs on soil nitrification processes was reported in other works, although the data varied widely. Some authors [28, 29] reported that application of phenanthrene at the level of 10 or 100 mg · kg^{-1} had no significant effect on nitrification. Ping and Tieheng [30] noted 6 % inhibition of nitrification in light soil contaminated with phenanthrene at the level of 250 mg · kg^{-1}. Sverdrup et al [12] observed 10 % decrease of soil nitrifying bacteria activity at the phenanthrene dose of 42 mg · kg^{-1}. Authors [12] noticed almost 60 % inhibition of nitrification after addition of Phen at the concentration ≥ 300 mg · kg^{-1}. In the same experimental conditions (one soil, C_{org} = 16.0 g · kg^{-1}, pH_{H_2O} = 6.2) pyrene exhibited lower toxic activity, the 10–20 % inhibition of NP occurred at the highest level of 3000 mg · kg^{-1} [12]. In the case of B[a]P the first significant effects for soil-nitrifying bacteria were recorded at 977 mg · kg^{-1} [2]. Remde and Hund [31] showed total inhibition of nitrification activity in soil contaminated with anthracene oil at concentration of 500 mg · kg^{-1}, at the same time the significant increase of the actual respiration rate was observed. Smreczak et al [15] noted that contamination of three soils (pH_{KCl} from 4.2 to 6.3; soil organic matter about 30 g · kg^{-1}) with Phen resulted in the 30–60 % inhibition

of nitrifying bacteria activity at hydrocarbon levels ≥ 100 mg · kg^{-1}. The similar effect of Phen was found in the studies of Klimkowicz-Pawlas and Maliszewska-Kordybach [32]. Higher sensitivity of NP parameter to phenanthrene contamination was reported by Maliszewska-Kordybach et al [1] in the studies involving 50 soils of different properties; in 60 % of the samples statistically significant decrease of RNP value corresponded to Phen concentration of 10 mg · kg^{-1}. Inhibition of NP was observed by Klimkowicz-Pawlas and Maliszewska-Kordybach [32] and Maliszewska-Kordybach et al [1] in soils characterised by low organic matter content (OM < 12 g · kg^{-1}).

The observed stimulation of microbial activity at low doses of PAHs (Table 2) is known in the literature as "hormesis" and describes over-reaction of organisms in response to small deviations from the physiological norm [22]. At the low levels (≤ 10 mg · kg^{-1}) of soil contamination with phenanthrene or pyrene the increase in the dehydrogenases activity [14], nitrification potential [1] and intensity of respiration [3] was noticed.

Relatively higher ecotoxic effect of phenanthrene, as compared to pyrene, can be explained by the properties of the hydrocarbons. Water solubility of phenanthrene (1300 μg · dm^{-3}) is about 10 times higher than solubility of pyrene [11]. This property may decide about higher bioavailability and thus toxicity of this compound to soil organisms. The toxicity of hydrocarbons can be predicted using the QSAR (*Quantitative Structure Activity Relationships*) model, describing the relationship between the biological activity and physical-chemical properties (eg solubility) of compounds [12, 19]. In soils freshly contaminated with Phen (condition of the experiment) the bioavailable fraction of Phen (24 h extraction with Tenax) is relatively high and may reach 90 % [15]. Pyrene exhibits higher sorption affinity to soils organic mater, as expressed by its octanol/water partition coefficient [19]. Stronger binding to soil organic and mineral fractions diminishes PAHs bioavailability and toxicity [2, 3, 10].

Soils properties exhibited moderate, although significant, influence on ecotoxic effects of tested PAH compounds. The most important parameter was soil acidity regulating conditions for nitrifying bacteria development and processes [6, 33]; soils of lower pH exhibited also lowest NP values (Table 1). The effect of phenanthrene and pyrene was significantly lower in the group of soils of lower acidity and higher organic matter content (Fig. 2), which confirms that sorption processes of PAHs by mineral and organic soil fractions reduce their bioavailability – and thus their toxicity – to microorganisms [3, 7, 10]. This is supported by stronger influence of soil properties on the ecotoxicity of pyrene as compared to phenanthrene – Table 3.

Besides influence on soil sorption abilities, the high acidity of soils can create additional stress to nitrifying bacteria and thus increase their susceptibility to the effect of contaminants [3, 34]. The problems of the relationships between soils properties and ecotoxic effects of PAHs towards soil nitrifying bacteria were discussed wider in the earlier studies [1, 3, 5].

The suitability and application of NOEC and LOEC values in ecotoxicity studies is questionable [1, 27, 35] due to their dependency on the conditions of the experiment, on the concentration pattern used and on the statistical procedure applied. Our study indicates on much lower LOEC values (1 mg · kg^{-1} for over 50 % of the data) as

compared to the EC_{20} parameters; these are considered to be realistic "lowest effect" values assuming 20 % standard deviations in most of the ecotoxicity studies [27].

The high applicability of the square root regression for the calculation of the EC_{20} and EC_{50} parameters in the case of phenanthrene ecotoxicity was already proved in the earlier studies [1]. In this study the square root regression was the best fit equation in 45–60 % of calculations – Table 4. The received values varied widely from as low as 8 mg · kg^{-1} of EC_{20} for pyrene in soils No 4 (acidic, C_{org} content < 10 g · kg^{-1}) to EC_{50} of 374 mg · kg^{-1} for phenanthrene in soil No 7 (neutral, C_{org} – 15.4 g · kg^{-1}) – Table 4. There are limited data in the literature on ecotoxic parameters of PAHs, especially in relation to microorganisms. The EC_{50} calculated by Sverdrup et al [12] corresponded to 250 mg · kg^{-1} for Phen (nitrification end-point, maximum applied concentration – 3000 mg · kg^{-1}), while EC_{10} for pyrene was 130 mg · kg^{-1}. Higher ecotoxicity parameters were reported by Maliszewska-Kordybach et al [1]; the EC_{50} for the effect of Phen on nitrification potential were within the range of 165–1670 mg · kg^{-1} (50 different soils, 7 days incubation, maximum applied concentration – 1000 mg · kg^{-1}). Relatively low PAHs toxicity towards soils microorganisms (evaluated on the basis of dehydrogenases activity) were observed in the studies of Klimkowicz-Pawlas and Maliszewska-Kordybach [13]; the EC_{50} values of 560–600 mg Σ2PAH · kg^{-1} were reported for soils freshly contaminated with a mixture of anthracene and pyrene.

Conclusions

Contamination of soils with PAHs caused adverse effects on the activity of nitrification bacteria. The nitrification potential was a good indicator of the reaction of this sensitive group of microorganisms to the presence of PAHs. The effect was related to compound properties and soil characteristic. Stronger inhibition of nitrifying bacteria activity was observed in the case of phenanthrene, characterised by high water solubility and bioavailability. Effect of pyrene, exhibiting stronger sorption capability towards soil organic and mineral fractions, was more related to soil properties. Light soils with low organic matter content and low biological activity were more susceptible to PAHs toxic effects. High acidity created additional stress enhancing negative reaction of nitrifying bacteria on chemical contamination with PAHs.

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POTENCJAŁ NITRYFIKACJI JAKO WSKAŹNIK EKOTOKSYCZNOŚCI WWA W GLEBACH ŚWIEŻO ZANIECZYSZCZONYCH NA PRZYKŁADZIE FENANTRENU I PIRENU

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Abstrakt: Celem pracy była ocena możliwości zastosowania potencjału nitryfikacji jako wskaźnika ekotoksyczności WWA w stosunku do mikroorganizmów glebowych w glebach świeżo zanieczyszczonych. Oddziaływanie dwóch modelowych związków z grupy WWA (fenantrenu i pirenu) badano w warunkach laboratoryjnych (inkubacja gleb przez 7 dni w temperaturze 20 ± 2°C). Do badań zastosowano materiał glebowy pochodzący z warstwy ornej (0–20 cm) ośmiu gleb, z terenów użytkowanych rolniczo oddalonych od źródeł emisji WWA. Materiał glebowy sztucznie zanieczyszczano fenantrenem lub pirenem w ilości 1, 10, 100 i 500 mg · kg^{-1} gleby. Zanieczyszczenie gleb WWA spowodowało zahamowanie aktywności bakterii nitryfikacyjnych, które wydają się być czułym wskaźnikiem obecności WWA. Oddziaływanie węglowodorów było uzależnione od właściwości gleb oraz właściwości związków; najsilniejsze hamowanie potencjału nitryfikacji odnotowano w glebach lekkich o małej zawartości substancji organicznej oraz małej aktywności biologicznej zanieczyszczonych fenantrenem (charakteryzującym się dużą rozpuszczalnością w wodzie i biodostępnością). Dodatkowym czynnikiem stresowym dla bakterii nitryfikacyjnych była duża kwasowość gleb, która zwiększała ich wrażliwość na oddziaływanie zanieczyszczeń typu WWA.

Słowa kluczowe: potencjał nitryfikacji, wielopierścieniowe węglowodory aromatyczne, fenantren, piren, parametry ekotoksyczności