Polish J. of Environ. Stud. Vol. 17, No. 5 (2008), 665-671

Original Research

Eco-Chemical Forest Soil Indices from a Forest Fire

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Received: 28 August, 2007
Accepted: 3 June, 2008

Abstract

Our paper presents results of investigations aimed at determining correlations between the content of polycyclic aromatic hydrocarbons (PAHs) and the enzymatic activity of a forest soil on an extensive area of forest damaged by a large fire 12 years ago. The investigations comprised experimental plots in the cultivations of Scots pine (*Pinus sylvestris* L.) and grey alder (*Alnus incana* L.) MOENCH on three sample surfaces on which different methods of soil preparation were applied. The statistical analysis of the obtained results clearly showed the inhibiting influence of the PAH content on the activity of dehydrogenases, acid phosphatase, alkaline phosphatase and protease, which indicated that the examined soils were loaded with PAHs to such a degree that it was dangerous to living organisms. A highly significant positive correlation was found between urease activity and PAH content.

Keywords: forest soils, fire-site, enzymatic activity, polycyclic aromatic hydrocarbons

Introduction

Within the framework of the monitoring project of the forest ecosystems undergoing regeneration since 1994, abundant data have been collected which make it possible to recognise the course of regeneration processes of different elements of the biosphere destroyed during that memorable fire from 1992 [1, 2]. The experimental areas are situated on a fire site in the central-northern part of the Notecka Primeval Forest, in Potrzebowice Forest District (52°53´N, 16°10´E).

In order to recognise and identify, in the regenerated forest ecosystems, the occurrence of a site drift following the application of regeneration operations and environmental factors, the authors employed communicative indices which describe the current eco-chemical condition of soils as well as indices which characterise trends of changes of site interrelationships.

The complex assessment of the eco-chemical condition of soils employs enzymatic indices which allow long-term monitoring and trend identification [3]. Changes in the soil enzymatic activity provide the earliest signals of changes in the intensity of live processes taking place in the environment, because many chemical compounds gain toxic or mutagenic traits following their metabolic transformations which occur only in living organisms [4]. The most important advantages resulting from the application of enzymatic tests include not only possibilities of performing serial analyses but, first and foremost, the capability of presenting, in a summary manner, the influence of many factors on the soil environment. Moreover, these tests allow carrying out the evaluation of parameters which are impossible to evaluate in any other way, e.g. elements of cell metabolism [5].

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The result of a complete destruction by fire of forest stands, organic matter and the biomass of microorganisms is a sudden influx to the soil of considerable quantities of polycyclic aromatic hydrocarbons (PAH) [6]. There is no data in the available literature on the subject concerning the effect of PAH contamination of soils from fire sites on the activity of soil enzymes. Most of the information on the subject is connected with obtaining such indices from laboratory experiments which fail to take into sufficient consideration both the defensive mechanisms which occur in natural soils and protect microorganisms and enzymes against environmental stresses, as well as current site conditions which determine the changing concentration of hydrocarbons in soils.

In order to ensure a continuous verification of diagnostic indicators of soil-forming processes as well as indicators of anthropogenic changes in the soils of the permanent sample plots on the fire site in Potrzebowice, relationships between the content of polycyclic aromatic hydrocarbons and soil enzymatic activity 12 years after the forest fire were determined. For the determination of soil enzymatic activity, the authors selected enzymes catalyzing the most important processes of organic matter transformations such as: transformations of the organic carbon compounds (dehydrogenases), phosphorus (acid phosphatase and alkaline phosphatase) and nitrogen (urease and protease).

Experimental Procedures

The investigated objects included forest soils on sample plots in the cultivations of Scots pine (*Pinus sylvestris* L.) and grey alder (*Alnus incana* L.) MOENCH situated on the biggest fire site from 1992 in Notecka Primeval Forest. The experiments included experimental plots on three surfaces (I, II and III) on which different methods of soil preparation were applied. The soil on plot I was altogether excluded from mechanical cultivation. Its preparation was confined to digging a hole (using a spade) where the seedling was to be planted. This method allows leaving the organic-mineral horizon intact and protection of the soil surface against erosion processes, which cause losses of organic compounds. In the case of plot II, full mechanical cultivation was applied, which comprised carrying out shallow ploughing to a depth of 30 cm, leading to a complete removal of the residues of organic horizon and its uniform mixture with the layer of mineral soil. On sample plot III, furrows were ploughed up every 1.5 m and their bottoms were additionally scarified with a subsoiler to a depth of 40 cm, and seedlings were planted on the belts formed in this way. In the last of the above-mentioned system, organic matter was distributed unevenly in the soil profile because the organic layer removed from the area of furrows was buried in inter-rows under part of the mineral soil. The experimental plots measuring 150 x 20 m, situated 20 m from one another, were each divided into 6 sub-plots of 25 x 20 m. Each of them was planted with one tree species out of a group of six tested by the Department of Silviculture of the Agricultural University in Poznań within the framework of a regeneration experiment [1]. Two-year old seedlings were used for the regeneration, which was carried out in the spring of 1994. In the studies presented in this paper, soil samples were collected only from under Scots pine (S) and grey alder (O). The control experimental plot (L) was selected in a natural forest complex neighbouring directly with the fire site. The examined soils represent rusty type of soils, of podzolic-rusty subclass and were formed from loose sands of fluvoglacial origin. They are characterised by a negligible proportion of the fraction with ∅ <0.02 mm.

In June 2004, soil samples were collected from five points of each of the experimental plots from the humus/eluvial horizon (AEes) in which enzymatic and chemical analyses were carried out in five replications. The following parameters were determined in the collected samples: activities of dehydrogenases [7], acid and alkaline phosphatases [8], urease [9] and protease [10] and the content of PAHs; pH reaction in $1 mol\cdot dm^{-3}$ KCl [ISO 10390]; organic carbon [ISO 14235]; crude nitrogen [ISO 13878], available potassium and phosphorus forms [11] and mineral forms of nitrogen $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ [ISO 14255]. PAHs were determined by the HPLC method [ISO 18287].

Statistical analyses were performed with the assistance of Statistica 6.0 PL and ARSTAT software programs.

Results and Discussion

Soils collected from the fire site were characterized by lower acidification than the control soil and the recorded differences ranged from 0.4 to 1.0 pH unit in $1 mol\cdot dm^{-3}$ KCl (Table 1). Within the fire site area, the soil acidification effect depended on the tree species which was connected, to a large extent, with the differences in the method of their nitrogen nourishment associated with the release of protons. Soils in pine stands were characterised by lower pH values (ranging from 0.3 to 0.7 pH units in $1 mol\cdot dm^{-3}$ KCl) in comparison with the soils on which alder was growing. The needle shed is more acid than the leaf shed and soil acidification is more apparent as the result of its decomposition. The methods of soil preparation employed to reclaim the fire site had no practical influence on changes in their reaction (Table 1).

Twelve years after the forest fire, the content of organic carbon and total nitrogen in the soils of the fire site was about two times lower than that in the soil from the control plot (Table 1). In the case of forest ecosystems, the main source of soil organic matter is the shed of plant material. The needle shed contains less nitrogen and more lignin than the broad-leafed shed. The coniferous substrate, rich in waxes, resins and lignin, is more resistant to degradation due to its high initial lignocellulosic index, which decides the rate of its microbiological transformation. The advantageous effect of the method of soil preparation during the process of regeneration of the fire site on the accumulation of total C_{org} and nitrogen was apparent in the case of the soil which was excluded from mechanical tillage (plot l). The observed differences in the content of these constituents in the soil were statistically significant (Table 1).

Table 1. Some chemical properties of the examined soils.

Object	pH	TOC	TN	C:N	P	K	N-NH4+	$N\text{-}NO_3^-$
	KCl	[g·kg⁻¹]			[mg·kg⁻¹]			
S I	3.7	12.96	0.58	22.3	7.60	9.94	27.51	5.64
S II	3.8	11.41	0.52	21.9	8.23	7.38	28.91	5.57
S III	3.6	11.32	0.54	20.9	7.55	8.52	26.78	5.03
O I	4.2	14.68	0.73	20.2	8.12	6.05	35.56	6.32
O II	4.1	14.06	0.65	21.6	8.47	5.90	39.77	6.75
O III	4.3	13.47	0.68	19.8	7.18	5.74	34.30	6.48
L	3.2	28.92	1.12	26.6	10.04	12.16	51.73	7.81
$LSD_{0.05}$		0.42	0.03	3.6	1.52	2.12	4.23	0.62

TOC – total organic carbon; TN – total nitrogen; P and K – available phosphorus and potassium forms

Table 2. Content [μg·kg⁻¹] of polycyclic aromatic hydrocarbons (PAHs) in soils.

PAHs	Object						
	S I	S II	S III	O I	O II	O III	L
Na	3522.7	794.2	719.2	410.5	6145.4	489.5	92.3
Ace	143.3	50.2	47.2	59.3	52.9	43.6	46.3
Fl	38.2	10.4	5.6	8.3	8.5	9.4	34.1
Phen	460.4	375.2	591.8	20.4	19.9	76.4	75.6
Ant	57.8	26.2	185.5	Not found	38.9	341.8	Not found
Fln	188.8	83.7	Not found	71.6	7.3	Not found	93.7
Pyr	152.8	88.7	Not found	39.7	Not found	Not found	90.7
BaA	66.0	75.3	Not found	40.0	Not found	Not found	66.0
Ch	15.6	46.4	Not found	9.7	9.7	Not found	Not found
BbF	74.3	92.1	7.9	55.6	8.0	21.6	47.3
BkF	11.0	106.6	7.1	38.1	5.0	32.3	Not found
BaP	238.5	207.0	Not found	114.6	11.3	Not found	665.1
DahA	25.6	102.9	6.0	11.6	5.4	20.6	16.6
BghiP	17.9	329.1	Not found	14.5	Not found	Not found	Not found
IndP	977.5	Not found	53.8	101.5	Not found	1119.8	Not found
15 PAHs	5990.4	2388.0	1624.1	995.4	6312.3	2155.0	1227.7

Na = naphthalene, Ace = acenaphthalene, Fl = fluorene, Phen = phenanthrene, Ant = anthracene, Fln = fluoranthene, Pyr = pyrene, BaA = benz[a]anthracene, Ch = chrysene, BbF = benzo[*b*]fluoranthene, BkF = benzo[*k*]fluoranthene, BaP = benzo[*a*]pyrene, DahA = dibenz[*a,h*]anthracene, BghiP = benzo[*ghi*]perylene, Ind = indeno[1,2,3-*cd*]pyrene.

Values of the C:N ratio in the soils damaged by the forest fire were significantly lower than in the soil from the control plot (Table 1).

The soils on the fire site were characterized by a significantly lower content of available forms of phosphorus and potassium (Table 1). The growing forest tree species and the methods of soil preparation applied during the reclamation process did not affect the content of these components in a significant way (Table 1).

The contents of mineral nitrogen forms ($N\text{-}NH_4^+$ and $N\text{-}NO_3^-$) in the soils from experimental plots situated on the fire site were significantly lower than in the control soil (Table 1). The quantities of these compounds in soils from the forest site were significantly higher on plots with grey alder than in the stands with Scots pine. This was probably associated with a different (depending on the tree species) rate of mineralization of the plant material fallout and the intensity of nutrient uptake by plants. The methods of soil

Table 3. Contribution [%] of individual PAHs in the pool of the determined PAHs in investigated soils in relation to the number of rings.

Object	Number of rings				
	2	3	4	5	6
S I	58.8	11.7	7.1	5.8	16.6
S II	33.2	19.4	12.3	21.3	13.8
S III	44.3	51.1	Not found	1.3	3.3
O I	41.2	8.8	16.2	22.1	11.7
O II	97.4	1.9	0.3	0.4	Not fund
O III	22.7	21.9	Not found	3.5	51.9
L	7.5	12.7	20.4	59.4	Not found

Table 4. Enzymatic activity of soils (ADh – dehydrogenases in cm^3 $H_2 \cdot kg^{-1} \cdot d^{-1}$, AFk – acid phosphatase and AFz – alkaline phosphatase in mmol $PNP \cdot kg^{-1} \cdot h^{-1}$, AU – urease in mg $N\text{-}NH_4^+ \cdot kg^{-1} \cdot h^{-1}$, AP – protease in mg $tyrosine \cdot g^{-1} \cdot h^{-1}$).

Object	DhA	AFk	AFz	AU	AP
S I	1.06	12.41	5.26	3.15	6.76
S II	1.42	8.02	5.38	2.01	7.29
S III	1.37	7.76	4.59	2.59	9.87
O I	1.64	28.29	9.03	1.49	12.86
O II	1.62	23.52	7.32	3.80	10.94
O III	1.67	34.17	6.98	2.74	12.43
L	4.94	48.93	23.16	1.85	15.21
$LSD_{0,05}$	0.19	3.22	1.58	0.74	1.02

preparation applied during the process of reclamation of the fire site failed to affect the contents of $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ in the soil in a significant way. The concentration of the ammonium form of nitrogen was several times higher than that of the nitrate form (Table 1).

Both the total PAH content as well as the group composition of these compounds were found to be considerably diversified (Table 2). The highest content of total PAHs was determined in the soils collected from S I and O II experimental plots (about 6000 $\mu g \cdot kg^{-1}$), while the lowest – in the soils from plot O I and control – L (about 1000 $\mu g \cdot kg^{-1}$). Two- and three-ring PAHs dominated in soils of the majority of the experimental objects in which they constituted from 50.0 to 99.3% of all the determined compounds (Table 3). The highest contents of 5- and 6-ring hydrocarbons were recorded in soils obtained from O III and L sample plots, where the proportion of these compounds among the determined PAHs exceeded 50% (Table 3). High contents of indeno[1,2,-cd]piren (1119.8 $\mu g \cdot kg^{-1}$) found in the soil from the OIII object and of benzo[a]piran (665.1 $\mu g \cdot kg^{-1}$) determined in the soil from the control plot deserve attention. Also soils from S II and O I objects were characterized by relatively high (over 30%) proportions of 5- and 6-chain hydrocarbons in the pool of the determined PAHs (Table 3).

The analysis of the obtained results failed to indicate unambiguously the effect of the method of soil preparation and tree species on the content of PAHs in the examined soils. The content of PAHs in soils changes with the passage of time and is influenced by the resultant of a number of biotic and abiotic processes taking place in the complex, multi-phase soil-plants-microorganisms-contaminations system and frequently the direction of these interactions is impossible to predict [12, 13]. PAH degradation in soils depends on many factors, including the amount and group composition of these contaminations, the desorption rate of the hydrocarbon fraction which are sorbed in the soil stronger, soil physico-chemical properties, and the capability of various groups of living organisms to uptake and decompose these compounds [12, 14].

The intensity of the PAH biodegradation is further influenced by "aging" of contaminants in the soil, i.e. by processes during which permanent combinations of contaminant-organic substance are developed, the encapsulation of contamination particles in the micro-pores of soil aggregates as well as the establishment of stable connections between contaminations and other soil components [13, 15]. In soils with "old" contamination, 5- and 6-chain hydrocarbons strongly sorbed by the solid fraction are dominant and

Table 5. Simple correlation coefficients (y = a + bx) between the activity of the examined enzymes and content of 15 PAHs.

	WWA	DhA	AFk	AFz	AU	AP
WWA	-	-0.50*	-0.47*	-0.49*	0.87**	-0.38*
DhA		-	0.79**	0.98***	-	-
AFk			-	0.85**	-	-
AFz				-	-	-
AU					-	-
AP					-	-

*** significant at α = 0.001; ** significant at α = 0.01;
* significant at α = 0.05.

they are potentially "not easily accessible" for the biotic elements of the soil environment. The sorption intensity is strongly influenced by the composition of organic matter. PAHs are sorbed most readily by humins, less readily by humic acids and least readily by fulvic acids [14]. The ambivalent influence of the plant species on the content of PAHs in soils was also reported by other researchers [13, 16]. Oleszczuk and Baran [17] found that the concentration of PAHs in rhizospheres clearly depended on plant species, although it exhibited a lower level than in the extra-rhizospheric soil. This confirms a positive impact of plants on PAH degradation due to a greater number of microorganisms in the rhizospheres.

The impact of the fire on soil enzymatic activity varied depending on the type of enzyme (Table 4). A particularly wide range of activity was obtained for dehydrogenases 1.06-4.94 cm^3 $H_2 \cdot kg^{-1} \cdot d^{-1}$) and phosphatases: acid (7.76-48.93 mmol $PNP \cdot kg^{-1} \cdot h^{-1}$) and alkaline (4.59-23.16 mmol $PNP \cdot kg^{-1} \cdot h^{-1}$).

The activity of dehydrogenases, examined phosphatases (acid and alkaline) and protease in the soils damaged by the forest fire was significantly lower (by about 3 and 1.5 times, respectively) than on the control surface. Reverse tendencies were found in the case of urease, which confirms the opinion that this enzyme is resistant to the activity of external factors and the only factor limiting its activity is the availability of the substrate – urea [18]. A significant decline of soil enzymatic activity 4 and 9 years after the forest fire was also reported by other researchers [19-21]. On the other hand, Januszek et al. [22] reported increased soil enzymatic activity 6 years after a forest fire. According to the above-mentioned researchers, sudden mineralization of considerable quantities of organic matter following complete burning of the forest supplied additional amounts of nutrients and increased soil pH and this created favourable conditions for the development of microorganisms. However, it is evident from the investigations carried out by Kaczmarek et al. [1] that the initial excess of nutrients occurring in soils of fire sites changes into their deficit with the passage of time as the nutrient contained in ashes found in the surface soil layers are either leached deeper into the soil profile with the intensity which depends on the soil conditions and atmospheric precipitation, or uptaken by plants. Our own investigations also determined significantly lower contents of organic carbon, total nitrogen, available forms of phosphorus and potassium as well as mineral forms of nitrogen in soils damaged by the forest fire in comparison with the soils obtained from the control surface (Table 1). These results indicate that the inactivation of the examined enzymes caused by the fire may, with time, generate a deficit of major nutrients. All biogen transformations taking place in the soil are stimulated by enzymes which control their transfer into forms available for microorganisms and plants as sources of energy and nutritive substances [5].

In the examined soils from the fire site the activity of dehydrogenases, acid and alkaline phosphatases as well as protease was found to be significantly lower in Scots pine stands in comparison with grey alder stands (Table 4). The activity of urease in the examined soils did not depend significantly on the tree species. The specific impact of individual tree species on soil enzyme activity is associated, among others, with the accumulation in the soil of specific substrates for enzymatic reactions [4]. No explicit influence of the method of soil preparation on the activity of the examined enzymes was determined (Table 4). The observed, distinctly favourable, impact of a complete resignation from mechanical soil cultivation on plot I (of relatively high organic carbon and total nitrogen contents) on its enzymatic activity was eliminated by the contamination of the soil environment by PAHs (Table 2).

The performed statistical analysis showed a reverse linear correlation between the activity of dehydrogenases, acid and alkaline phosphatases as well as protease and the content of the sum of the assayed polycyclic aromatic hydrocarbons (Table 5). In the case of urease, a highly significant positive correlation was found with PAH content. Reduced biomass of soil microorganisms and of enzyme activity in soils contaminated with PAHs were reported in many investigations [23-25]. Some researchers noticed a stimulating influence of these pollutants on soil biological activity which, in their opinion, was the effect of chemically induced selection and adaptation of microorganisms to the contaminated environment. The noxious influence of PAHs on soil microorganisms and natural biochemical processes strongly depend on the concentration of these compounds and the duration of their action as well as on soil conditions [26-28].

Recapitulating the obtained research results, it should be stated that, after 12 years, the process of regeneration of the soils damaged by the forest fire was not completed. Values of simple correlation coefficients between the activity of soil enzymes and PAH content in the examined soils indicate that the loading of these soils with PAHs remained at a level dangerous for living organisms. The contamination of the soil environment with PAHs is a long-term pollution because, as a result of processes of biological desorption, these compounds are released gradually from permanent bonds such as: contaminant – organic matter or connections between contaminants and other soil constituents [13, 27]. At the same time, it should be emphasised that as an open system to which many different substances enter or leave simultaneously, soil is constantly exposed to the activity of natural and anthropogenic factors. This indicates the need to continue investigations on changes in biochemical parameters from the point of view of complex interrelationships with environmental factors and long-term effects of reclamation operations. This should make it possible to maintain forest integrality and realize the strategy of its sustainable development.

Conclusions

1. The determined activities of dehydrogenases, acid and alkaline phosphatases and protease, as well as the content of organic carbon, total nitrogen and available forms of phosphorus and potassium and mineral forms of nitrogen in the examined soils of the fire site, were significantly smaller than in soils from the control plot (outside the fire site). This indicates that the process of reclamation of soils damaged by the forest fire was not completed after 12 years.
2. High enzyme inactivation caused by fire can lead to shortages of major nutrients in the examined soils.
3. The performed statistical analyses revealed explicitly the inhibiting impact of PAHs on the activity of dehydrogenases, acid and alkaline phosphatases and protease, which indicates that the load of the examined soils with PAHs remains on a level which is hazardous to living organisms.
4. The influence of the fire on soil enzymatic activity varied depending on the type of enzyme. In the case of urease activity, a highly significant positive correlation was found with PAH content. This indicates that reliable soil quality assessment can be achieved by simultaneous investigations of the activity of many enzymes, which allow us to register changes in specific capabilities of the soil complex taking place under the influence of stress conditions.
5. It is evident from the performed investigations that the employed enzymatic tests also provide information about changes taking place in the soil, resulting from the tree species and the way of soil, preparation. This, in turn, allows a comprehensive recognition of the course of the regeneration processes of forest ecosystems destroyed by fires and may be utilized to elaborate optimal concepts of management of extensive forest fire sites.

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