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FORMATION OF ADENYLATE ENERGY CHARGE (AEC) VERSUS THE FLUORINE CONTENT IN FOREST SOIL IN THE AREA AFFECTED BY EMISSION FROM POLICE CHEMICAL PLANT

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Abstract

Activity of most enzymes of the key-metabolic pathways depend on the content of adenine nucleotides, such as ATP, ADP and AMP, in cells. Based on the level of these nucleotides, the adenylate energy charge (AEC) was defined as AEC = ([ATP] + 0.5[ADP]) : ([ATP] + [ADP] + [AMP]). Theoretically, AEC values can range from 0 to 1 and represent the physiological state of a soil microbial population.

Soil microorganisms and the enzymes they secrete are connected with biological processes which form soil fertility in all ecosystems, including forests. Forests are such ecological systems which are an integral complex and their dysfunction could be caused by permanent influence of anthropogenic factors, including industrial emission of gases and dusts.

The aim of this study was the determination of changes in adenylate energy charge values and fluorine content in the humus layer of forest-podsol soils affected by the emission of Police Chemical Plant. During a year, soil samples were taken five times (in October 2007, February, April, June and September 2008) from five different sites Wkrzańska Forest near Węgornik, Tatynia, Tanowo, Trzeszczyn and Mścięcino. In the samples, concentration of fluorine, both water-soluble (extracted by 0.01 M $CaCl_2$) and potentially accessible to plants (extracted by 2 M $HClO_4$), was assayed by potentiometry. Additionally, the content of adenine nucleotides was assayed by chromatography. Based on the content of nucleotides, adenylate energy charge values in soil were calculated. The AEC values and fluorine content in soil depended on a distance from the emitter and the dates on which the samples were taken. In order to determine the relationships between the fluorine content and AEC values, Pearson's correlations coefficients were calculated. Between

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the fluorine (both, water-soluble and plant available) content and AEC values there was a signifficant negative correlation, which could mean that AEC is a very good indicator of the fluorine content in soil.

Key words: fluorine, adenylate energy charge, soil, forest.

KSZTAŁTOWANIE SIĘ ŁADUNKU ENERGETYCZNEGO KOMÓRKI NA TLE ZAWARTOŚCI FLUORU W GLEBACH LEŚNYCH Z TERENÓW OBJĘTYCH EMISJĄ ZAKŁADÓW CHEMICZNYCH POLICE S.A.

Abstrakt

Aktywność większości enzymów kluczowych szlaków metabolicznych zależna jest od zawartości w komórce nukleotydów adeninowych: ATP, ADP i AMP. Na podstawie zawartości tych nukleotydów zdefiniowano pojęcie ładunku energetycznego komórki (AEC), jako: AEC = ([ATP] + 0,5[ADP]) : ([ATP] + [ADP] + [AMP]). Teoretycznie wartości AEC mieszczą się w przedziale od 0 do 1 i obrazują stan fizjologiczny populacji mikroorganizmów glebowych.

Właśnie z mikroorganizmami i wydzielanymi przez nie enzymami są związane biologiczne procesy kształtujące żyzność gleby we wszystkich ekosystemach, w tym ekosystemach leśnych. Lasy są układami ekologicznymi stanowiącymi pewną całość pod względem przyrodniczym, a zachwianie ich funkcjonowania może następować w wyniku trwałego wpływu czynników antropogenicznych, w tym emisji przemysłowych o charakterze gazowym i pyłowym.

Celem badań było określenie zmian wielkości ładunku energetycznego komórki oraz zawartości fluoru w warstwie próchnicznej leśnych gleb bielicowych objętych emisją Zakładów Chemicznych Police S.A. W ciągu roku pobrano pięciokrotnie (październik 2007, luty, kwiecień, czerwiec i wrzesień 2008) próbki glebowe z terenów Puszczy Wkrzańskiej w okolicach Węgornika, Tatyni, Tanowa, Trzeszczyna, Mścięcina oraz oznaczono w nich potencjometrycznie zawartość fluoru rozpuszczalnego (ekstrahowanego 0,01 M $CaCl_2$) i potencjalnie dostępnego dla roślin (ekstrahowanego 2 M $HClO_4$), a także chromatograficznie zawartość nukleotydów adeninowych. Na podstawie zawartości nukleotydów obliczono wielkość ładunku energetycznego komórki w glebie. Wielkość AEC oraz zawartość fluoru w glebie była determinowana odległością od emitora oraz terminem pobierania próbek. W celu określenia zależności między zawartością fluoru a wielkością AEC obliczono współczynniki korelacji liniowej Pearsona. Na ich podstawie stwierdzono istotną ujemną zależność między zawartością w glebie fluoru (zarówno rozpuszczalnego, jak i potencjalnie dostępnego dla roślin) a wielkością ładunku energetycznego komórki, co może świadczyć o tym, iż AEC jest bardzo dobrym wskaźnikiem zawartości fluoru w glebie.

Słowa kluczowe: fluor, ładunek energetyczny komórki, gleba, ekosystemy leśne.

INTRODUCTION

Forest ecosystems as ecological structures are an integral complex. They include organisms and their abiotic environment. Dysfunction of their functioning could be caused by permanent influence of anthropogenic factors, including industrial emission of gases and dusts (TELESIŃSKI et al. 2008).

Soil is one of the environmental elements which are strongly affected by pollution. Soil is highly biologically active and depressing soil activity indicates soil contamination. The activity of most enzymes of key-metabolic pathways depends on the content of adenine nucleotides, such as adenosine tri- (ATP), di- (ADP) and monophosphates (AMP)in cells. These three adenine nucleotides give information about adenylate energy charge, which was defined by ATKINSON and WALTON (1977) as ([ATP] + 0.5 [ADP]) : ([ATP + [ADP] + [AMP]).

Theoretically, AEC values can range from 0, corresponding to a totally dephosphorylated adenine nucleotide pool of heavily impaired microorganisms, to 1, corresponding to a completely phosphorylated adenine nucleotide pool of viable microorganisms under optimal growth conditions (WIESE, SEYDEL 1995). AEC values above 0.8 are the evidence of intense growth; AEC values between 0.5 and 0.7 represent cells incapable of reproduction and AEC values under 0.4 occur in a dying microorganisms population (JOERGENSEN, RAUBUCH 2002). Fluorine is a chemical element especially dangerous to the environment. It belongs to the elements defined as having a small range of safe levels (EVDOKIMOVA 2001). Nevertheless, it activates some enzymes. More often, however, it contributes to disturbances of biochemical functions in cells.

The aim of study was to determine changes in the fluorine content and AEC values in forest-podsol soils affected by emission from Police Chemical Plant and located at different distance from the source of emission, and to assess the relationship between the fluorine content and AEC.

MATERIAL AND METHODS

The material for analyses consisted of soil samples taken from the humus layer of forest- podsol soils affected by the emission of Police Chemical Plant. During a year, soil samples were taken five times (in October 2007, February, April, June and September 2008) from five different sites in Wkrzańska Forest near Węgornik, Tatynia, Tanowo, Trzeszczyn and Mścięcino. The location of the sites is presented in Table 1. The organic carbon content in these soils was between 10-12%.

In the soil samples, concentration of water-soluble fluorine (extracted by 0.01 M $CaCl_2$) and plant available fluorine (extracted by 2 M $HClO_4$) was assayed by potentiometry with a pH/mV Orion 920 A device with an ion-selective fluorine electrode, according to LARSEN and WIDDOWSON (1971) and according to OGOŃSKI and SAMUJŁO (1996) as modified by NOWAK and KURAN (2000).

Measurements of adenine nucleotides and calculations of the adenylate energy charge were made according to the procedure elaborated by BAI et al.

Table 1

Location of sampling sites

Site	Distance from Police Chemical Plant (km)	Direction
Tatynia	2.5	W
Trzeszczyn	3.5	SW
Mścięcino	4.5	S
Tanowo	6.5	SW
Węgornik	10.0	W

(1988) as described by DYCKMANS and RAUBUCH (1997). Dimethylsulphoxide (DMSO), Na_3PO_4 (10 mM) buffer + EDTA (20 mM) at pH 12 were used as extractants. After derivatisation with chloracetaldehyde, adenine nucleotides were determined by HPLC. The separation was carried out on a Hypersil C18 5µ ODS (250 × 4.6 mm) column. Chromatography was performed isocratically (2 cm^3 min^{-1}) with 50 mM ammonium acetate buffer containing 1 mM EDTA and 0.4 mM TBAHS mixed with methanol (90 : 10 v : v) as a mobile phase. Fluorometric emission was measured at a wavelength of 410 nm with 280 nm as the excitation wavelength. The AEC was calculated as ([ATP] + 0.5[ADP]) : ([ATP] + [ADP] + [AMP]).

The results were processed statistically using two-way analysis of variance. The least significant differences (LSD) were determined by Tukey's test at α = 0.05. In order to determine relationships between the fluorine content and AEC values, the results were analysed statistically using Pearson's linear correlation coefficients. Statistical calculations were carried out with the use of Statistica 8.0 programme.

RESULTS AND DISCUSSION

During the study, significant differences of water-soluble and plant available fluorine in forest soils were noticed for all sampling dates and sites. The water-soluble fluorine content was in the range of 1.259 to 10.272 mg kg^{-1}d.w. soil, and the content of fluorine potentially available to plants was in the range from 59.3 to 302.1 mg kg^{-1}d.w. soil (Table 2). These values are similar to the fluorine content in garden soils affected by emissions from Police Chemical Plant, which was determined by KŁÓDKA et al. (2008). The smallest mean content of fluorine, both water-soluble and potentially accessible to plants, was recorded near Trzeszczyn, in the SW direction. The largest water-soluble fluorine content was found in soil samples taken from

Table 2

Fluorine content in forest soil near Police Chemical Plant (mg kg^{-1} d.w. soil)

Water-soluble fluorine in soil						
Date (*A*)	Tatynia	Trzeszczyn	Mścięcino	Tanowo	Węgornik	mean
	sites (*B*)					
9.10.2007	4.410	4.290	3.771	4.422	5.211	4.421
27.02.2008	1.728	1.598	1.259	1.603	1.541	1.546
23.04.2008	8.243	6.948	9.208	8.751	10.272	8.684
16.06.2008	1.161	1.132	3.792	1.699	3.637	2.284
27.09.2008	1.898	2.280	3.947	2.262	3.750	2.827
Mean	3.488	3.250	4.395	3.747	4.882	3.953
$LSD_{0.05}$	*A* = 0.302 *B* = 0.248			A × B = 0.602 B × A = 0.561		
Fluorine potentially accessible to plants						
Date (*A*)	sites (*B*)					
	Tatynia	Trzeszczyn	Mścięcino	Tanowo	Węgornik	mean
9.10.2007	136.3	145.1	156.5	145.2	137.9	144.2
27.02.2008	84.8	72.2	59.3	74.6	62.9	70.8
23.04.2008	292.0	237.8	282.7	266.6	302.1	276.2
16.06.2008	163.5	197.2	234.7	233.6	211.9	208.2
27.09.2008	196.3	178.4	297.7	264.6	229.8	233.4
Mean	174.6	166.1	206.2	196.9	188.9	186.5
$LSD_{0.05}$	*A* = 5.921 *B* = 4.968			A × B = 11.82 B × A = 11.21		

the farthest site from the emitter (Węgornik, 10 km W), whereas fluorine potentially accessible to plants was the most abundant in Mścięcino, which is remote from Police Chemical Plant. Nevertheless, results of many studies have shown that fluorine content in soil diminishes with a growing distance from the emitter of this element (FRANZARING et al. 2006). A reverse relationship has been determined for the water-soluble fluorine content. However, in the present study, same as in the investigations completed by KŁÓDKA et al. (2008), no relationship has been found between the content of fluorine potentially accessible to plants and the distance from Police Chemical Plant.

Fluorine content in forest soil was the highest in spring, which could have been caused by decay of shed leaves and needles, which accumulated fluorine from air (FRANZARING et al. 2006). Afterwards, the fluorine content in forest soil decreased. KŁÓDKA et al. (2008) showed that the highest fluorine

Table 3

AEC values in forest soil near Police Chemical Plant

Date	Sites				
	Tatynia	Trzeszczyn	Mścięcino	Tanowo	Węgornik
9.10.2007	0.791	0.800	0.782	0.822	0.811
27.02.2008	0.889	0.908	0.894	0.903	0.882
23.04.2008	0.643	0.760	0.657	0.642	0.589
16.06.2008	0.922	0.932	0.789	0.899	0.827
27.09.2008	0.878	0.838	0.811	0.856	0.840

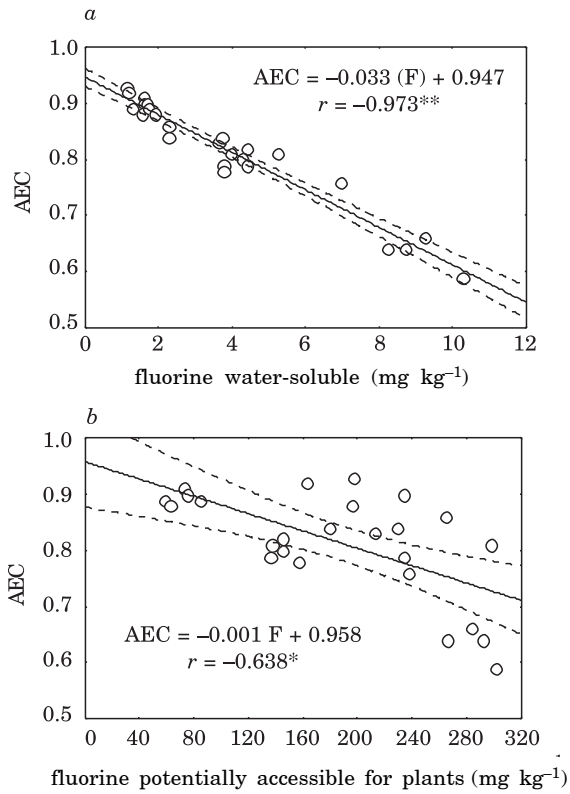

Fig. 1. Relationship between content of fluorine: water-soluble (*a*) and potentially accessible to plants (*b*), and adenylate energy charge in forest soil near Police Chemical Plant (* – significant at $p = 0.05$, ** – significant at $p = 0.01$)

content in garden soils attributable to emission from Police Chemical Plant was in June, declining during the plant growing season. This might have been caused by the fact that fluorine was taken up by growing plants.

The adenylate energy charge (AEC) values in the humus layer of forest soils near Police Chemical Plant varied from 0.589 to 0.932 (Table 3) and depended on the fluorine content. Many authors showed that AEC decreased in the presence of xenobiotics in soil, for example heavy metals (CHANDER et al. 2001) and pesticides (LEHR et al. 1996, NOWAK et al. 2006).

The calculated coefficients of Pearson's linear correlation showed a significant negative relationship between the fluorine content in soil and adenylate energy charge (Figure 1). However, the correlation coefficients between the water-soluble fluorine content and AEC were significant at $p = 0.01$, and between the content of fluorine potentially accessible to plants and AEC were significant at $p = 0.05$. Thus, AEC could be a very good indicator of fluorine (especially in its water-soluble form) content in soil. Other biochemical parameters could be used for bioindication of soils contaminated with fluorine. NOWAK et al. (2005) showed that inhibition of the activity of phosphatases, β-glucosidase and dehydrogenase was significantly positively correlated with fluorine content in soil. Earlier studies by other authors showed that soil enzymatic activity, especially that of acid phosphatase, could also be a very good indicator of soil contamination with this element (TELESIŃSKI et al. 2008).

CONCLUSIONS

1. Fluorine content in the humus layer of forest-podsol soils depended on the distance from Police Chemical Plant and increased with a growing distance from the emitter.

2. Adenylate energy charge values depended on the fluorine content in soil, especially in its water-soluble form, and significantly decreased with an increasing concentration of this element.

3. Adenylate energy charge could be a very good indicator of fluorine content in forest soil.

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