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THE USE OF ENZYMATIC TESTS FOR QUALITY ASSESSMENT OF SOILS IN REMEDIED POST-INDUSTRIAL AREAS

ZASTOSOWANIE TESTÓW ENZYMATYCZNYCH DO OCENY JAKOŚCI GLEB NA REKULTYWOWANYCH TERENACH POPRZEMYSŁOWYCH

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Streszczenie. W pracy zastosowano komunikatywne wskaźniki enzymatyczne do oceny jakości gleb na rekultywowanych terenach poprzemysłowych. Badaniami objęto gleby na terenach pokolejowych w Lublinie i Radomiu oraz gleby na rekultywowanych terenach przy Zakładach Azotowych „Puławy” S.A. i na składowisku popiołów przy Zespole Elektrowni „Dolna Odra” S.A. w Nowym Czarnowie. Przeprowadzone badania wykazały wysoką inaktywację biologiczną gleb na terenach pokolejowych. W przypadku gleb na terenach przy Zakładach Azotowych „Puławy” i na składowisku popiołów w Nowym Czarnowie uzyskane wyniki wskazują na efektywność zastosowanych systemów rekultywacji. Zaproponowane komunikatywne wskaźniki enzymatyczne, odzwierciedlające metabolizm ekosystemu glebowego, pozwalają na szybką ocenę jakości i zdrowotności gleb, a także umożliwiają kwantyfikację ekologicznych efektów realizacji zastosowanego systemu rekultywacji bądź programu ochrony ekosystemów na terenach poprzemysłowych.

Key words: soil, post-industrial area, land reclamation, enzymatic activity.
Słowa kluczowe: gleba, tereny poprzemysłowe, rekultywacja, aktywność enzymatyczna.

INTRODUCTION

The post-industrial and post-railway areas are characterised by considerable contamination of the soil environment and require remediation. For many years, the railway has been the dominant transport means for different kinds of goods, including materials which are toxic and dangerous to the natural environment. Various types of treatment aimed at restoring soil bioactivity are utilised in bioremediation of degraded lands, such as mechanical treatment, regulation of soil pH, topsoil liming, the use of comminuted brown coal, peat, straw, sawdust, green fertilisers, compost, sewage sludge, growing of legume plants, mineral and organic fertilisation, and afforestation of poor soils (Karczewska 2008). Railway grounds form a part of the so-called closed areas and are protected by law as areas important to national defence. They are also subject to legal provisions concerning landscaping and land management along railway tracks (Gleisenstein et al. 2002), which restricts the possibilities

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of effective soil remediation. The loss of soil identity in post-industrial areas deprives them of their value, posing a problem and a serious financial burden for local authorities (Środulska--Wielgus 2007). Effective soil remediation in post-industrial areas is essential for both environmental protection and sustainable development policy. The use of enzymatic tests to evaluate the quality of soils and the effectiveness of the applied post-industrial soil remediation methods enables the environmental risk to be assessed without the need to identify multiple compounds, which is highly justified economically. Determination of the xenobiotic content in the soil does not reflect the actual ecotoxicological hazard connected with their presence in the environment. It stems from the fact that amongst approximately one hundred thousand chemical compounds the toxicity of which has been documented, less than 170 are routinely analysed. The rest, i.e. 99%, is not monitored (Wolska et al. 2006).

Moreover, many chemical compounds acquire toxic or mutagenic properties after metabolic transformations that take place in living organisms. Metabolites are often characterised by even more harmful properties than the contaminants from which they originate (Meyer and Steinhart 2001). While evaluating the quality of soils, it is important to assess the level of contamination that can be tolerated without any negative impact on soil organisms and plants. The enzymatic activity of soils reflects the contamination level of the environment that is hazardous to living organisms (Bielińska et al. 2014). The purpose of this study was the assessment of the ecochemical condition of soils in post-industrial areas. This assessment was made basing on the determination of the activity of several soil enzymes.

MATERIAL AND METHODS

The object of testing was soil material collected from the humus layer (depth 0–30 cm) of sodded areas located in northern areas of the city of Lublin (near the former Northern Railway Station) and in the central parts of Radom (separated lot no. 3/62 situated at ul. Bieliny Prażmowskiego), and for reference: from park areas located at the outskirts of these cities, with similar physiographic conditions but not subjected to the direct effects of anthropogenic contamination. All areas selected for the tests were characterised by typologically transformed soils with the granulometric composition of loam. The analysed soil sample was made by averaging 5 samples taken from each area.

The soil tests also included remediated post-industrial areas, i.e.: humus layers (0–30 cm) at the tested sites near ZA "Puławy" SA and top fertile layers of artificially formed overburden (0–30 cm) on the coal ash dumpsite at ZE "Dolna Odra" SA in Nowe Czarnowo.

The research works near ZA "Puławy" SA were conducted in test areas afforested in 2002 with 1-year Scots pine (*Pinus sylvestris* L.) – mycorrhized and non-mycorrhized saplings. The test sites were situated in the south-western part of the industrial complex. The control object was a non-forested area located nearby. The soils present there can be defined as anthropogenic post-podsol soil that is the result of transformation of arenosol formed from fluvioglacial sand. Similarly as in the case of soils from the post-railway areas, the analysed soil sample was prepared by averaging 5 specimens taken from each area.

At the coal ash dumpsite in Nowe Czarnowo, the research was carried out based on the remediation experiment. The experiment was set up in 2003, using the split experimental lots method in 5 replications. The object of this research was the top layer of the overburden, made of natural soil commonly present in the Odra valley, with the grain composition of loose sand, compost made using the GWDA method, and fermented sewage sludge with the following composition (in % of dry matter): sludge 70%, straw 15%, urban greenery waste 15%, at a 1 : 1 : 2 ratio, as well as the control lots, on which the overburden was formed of natural soil only. The analysed soil sample was made by averaging the samples taken from each lot.

The laboratory test soil samples from the selected areas were collected in the second half of September 2015, during a period of stable weather. The dates of soil sampling were scheduled for a period when the soil was in a dynamic equilibrium which ensured that moderate intensity of biochemical processes was maintained. Optimally selected soil sampling dates are essential when testing the activity of biochemical processes in a soil environment (Šarapatka 2002; Bielińska et al. 2014). Enzymatic analyses during a period of intense micro-flora growth, resulting for example from increased microbiological activity occurring within 1 to 2 days after intense precipitations, especially ones that follow a period of drought, present the results of temporary, heightened biochemical activity of the soil that deviates from its average level (Bielińska and Mocek-Płóciniak 2009).

Activities of the following enzymes: dehydrogenases (Thalmann 1968), phosphatases (Tabatabai and Bremner 1969), ureases (Zantua and Bremner 1975) and proteases (Ladd and Butler 1972)] were determined in the soil material samples, as well as basic chemical features: pH in 1 mole · dm^{-3} KCl (ISO 10390), organic carbon (ISO 14235) and total nitrogen (ISO 13878). The tested enzymes participate in the biochemical circulation of carbon (dehydrogenases), nitrogen (urease and proteases) and phosphorus (phosphatases) in ecosystems.

All the determinations were made in three parallel replications. The results obtained were statistically assessed (Maliński 2004; StatSoft 2006). The significance level used most commonly in environmental tests e.g. $\alpha = 0.05$ (with event probability $p = 0.95$) was assumed.

RESULTS AND DISCUSSION

Soils in the studied areas along railways were characterised by high pH values (pH_{KCl} : : 6.8–7.4) – Table 1. Alkalisation of these soils was related to the character of contaminants flowing thereto in the areas along the railways, such as i.a.: alkaline dusts, heavy metal ores, agents used for snow removal from streets, as well as to the presence of calcium carbonate of anthropogenic origin that was introduced to the soils during railway transport of construction materials or during repair works (Greinert 2003). On the other hand, control soils (from parks located at city outskirts) had slightly acidic reactions: pH_{KCl} 6.2–6.5 (Table 1).

The soils at the ZA "Puławy" SA site were had a highly acidic reaction, with a pH value in 1 mole KCl · dm^{-3} from 3.5 in the control area up to 4.1 in afforested areas. The strong acidity of the analysed soils is related to long lasting intense nitrogen emission. In ecosystems of this type, the nitrogen circulation processes contribute significantly to acidification of the soil environment (Kurek 2002). With elevated inflow of nitrogen to forested soils, the release of H^+ protons due to nitrogen fertilisation is not balanced by their fixation during mineralisation of plant material, which contributes to revealing the secondary effects of soil acidification (Bielińska et al. 2009).

Table 1. Content of organic carbon (C in g · kg^{-1}), total nitrogen (N in g · kg^{-1}), ratio C : N and pH in soils
Tabela 1. Zawartość węgla organicznego (C w g · kg^{-1}), azotu ogółem (N w g · kg^{-1}), stosunek C : N i pH gleb

Object – Obiekt	pH_{KCl}	C	N	C : N
Lublin – post-railway area – teren pokolejowy	6.8	24.49^{bc}	1.63^{b}	15.0^{b}
Lublin – control area – powierzchnia kontrolna	6.2	19.92^{b}	2.28^{b}	8.7^{a}
Radom – post-railway area – teren pokolejowy	7.4	28.32^{c}	1.74^{b}	16.2^{b}
Radom – control area – powierzchnia kontrolna	6.5	20.84^{b}	2.51^{b}	8.3^{a}
Zakłady Azotowe – mycorrhized saplings – sadzonki mikoryzowane	4.1	1.80^{a}	0.12^{a}	15.0^{b}
Zakłady Azotowe – non-mycorrhized saplings – sadzonki niemikoryzowane	4.1	1.62^{a}	0.11^{a}	14.7^{b}
Zakłady Azotowe – control area – powierzchnia kontrolna	3.5	0.82^{a}	0.05^{a}	16.4^{b}
Nowy Czarnów – overburden layer – warstwa nadkładu	7.4	3.54^{a}	0.26^{a}	13.6^{b}
Nowy Czarnów – control area – powierzchnia kontrolna	7.8	1.12^{a}	0.08^{a}	14.0^{b}

Values in the column followed by the same letter do not differ significantly at $p < 0.05$; t-test – Wartości w kolumnie oznaczone tą samą literą nie są istotnie różne przy $p < 0,05$; test – "t".

In the hard coal ash dumpsite in Nowe Czarnowo, after 12 years since the start of the remediation experiment, the alkaline ash fallout has led to the natural soil at the control lots acquiring a basic reaction. However, the overburden layer was nearing neutral pH (Table 1), under the effect of intense biological activity (Table 2).

Table 2. Enzymatic activity of soils
Tabela 2. Aktywność enzymatyczna gleb

Object – Obiekt	DhA	PhA	UA	PA
Lublin – post-railway area – teren pokolejowy	0.82^{a}	5.40^{a}	3.11^{a}	1.26^{a}
Lublin – control area – powierzchnia kontrolna	4.25^{d}	16.63^{d}	14.95^{c}	9.28^{d}
Radom – post-railway area – teren pokolejowy	0.66^{a}	5.12^{a}	2.57^{a}	1.09^{a}
Radom – control area – powierzchnia kontrolna	4.04^{d}	15.41^{d}	13.84^{c}	8.74^{d}
Zakłady Azotowe – mycorrhized saplings – sadzonki mikoryzowane	2.68^{c}	11.97^{c}	12.62^{c}	4.72^{c}
Zakłady Azotowe – non-mycorrhized saplings – sadzonki niemikoryzowane	1.45^{b}	8.04^{b}	8.29^{b}	2.15^{b}
Zakłady Azotowe – control area – powierzchnia kontrolna	0.84^{a}	5.62^{a}	3.02^{a}	1.49^{a}
Nowy Czarnów – overburden layer – warstwa nadkładu	4.04^{d}	15.46^{c}	12.94^{c}	8.82^{d}
Nowy Czarnów – control area – powierzchnia kontrolna	1.26^{b}	10.22^{b}	7.40^{b}	2.12^{b}

DhA – dehydrogenases activity in mmol TFF kg^{-1} · h^{-1} – aktywność dehydrogenaz w mmol TTF kg^{-1} · h^{-1}, PhA – phosphatases activity in mmol PNP · kg^{-1} · h^{-1} – aktywność fosfataz w mmol PNP · kg^{-1} · h^{-1} , UA – urease activity in mg N-NH_4^+ · kg^{-1} · h^{-1} – aktywność ureazy w mg N-NH_4^+ · kg^{-1} · h^{-1}, PA – proteases activity in mg tyrosine · kg^{-1} · h^{-1} – aktywność proteazy w mg tyrozyny · kg^{-1} · h^{-1}.
Values in the column followed by the same letter do not differ significantly at $p < 0.05$, t-test – Wartości w kolumnie oznaczone tą samą literą nie są istotnie różne przy $p < 0,05$, test – "t".

In soils in the areas near the railway system, the resources of organic carbon were significantly higher than in the control soils from the selected urban parks. The opposite trends were observed for the total nitrogen content (Table 1). A high content of organic carbon in the soils located along railway tracks was related to the railway transport of hard and brown coal, asphalt and other products containing considerable amounts of this component, e.g. car tires. A factor that modified the content of Corg. could be the amount of this component being deposited on the railway soils with dry or wet fallout due to the concentration of industrial plants near a railway station, as well as to the presence of the still functioning ZNTK (Rail Rolling Stock Repair Workshops) situated in the vicinity of large railway junctions. The locations of industrial plants were dictated by an easy access to railway lines, stations and loading sidings (Przemysł Radomia 1970; Bochyński 2007). The increased inflow of organic carbon of anthropogenic origin, so called black carbon (Oleszczuk 2007), to the soil environment in the post-railway track areas resulted in a significantly wider range of values for the C : N ratio than in the control soils (Table 1).

In the soils near ZA "Puławy" SA, the content of organic carbon and total nitrogen was twice higher in the forested areas than in the soil from control lots (Table 1). The increase in organic C and total N in the soil from forested areas was related to the decomposition of plant material and the release of these components through tree roots. The amount of organic carbon released to the rhizosphere by plants may equal 40% of the total dry matter produced by a plant (Domżał and Bielińska 2007).

At the ash dumpsite, the fertile soil layer (an overburden containing admixtures rich in organic matter) was characterised by a noticeably higher content of organic carbon and total nitrogen in comparison with the control soil (Table 1), albeit after 12 years since the start of the soil remediation system, these amounts were two times lower than during the initial stage of the experiment (Bielińska et al. 2005). Further loss of organic matter may lead to gradual elimination of some functions of the soil environment, and consequently to their decay to such a degree that would require the remediation process to be repeated in the future.

The study revealed high inactivation of the analysed enzymes in soils in post-railway areas, at the activity level of enzymes assayed in the degraded, non-remediated soil near ZA "Puławy" SA (the control soil) – Table 2. These results indicate biological degradation of the soils in question, manifesting itself through an inhibition of natural biochemical processes and leading to a number of undesirable changes. The reason for this condition is heavy contamination of soils in the areas along railway tracks (Uherek et al. 2010). It is worth emphasising that railroad sleepers have been protected for decades with toxic, carcinogenic creosote. These areas are located within cities and are a potential risk to residents' health. Effective remediation of post-railway areas should form an integral part of any comprehensive strategy of urban landscape protection and utilisation. In the opinion of Wielgus (2007), regardless of localised positive examples, the tendency of post-industrial landscapes in Poland, including the post-railway ones, to devaluate is clearly escalating.

In the forested areas near ZA "Puławy" SA, the enzymatic activity of soils was significantly higher than in the control area (table 2). The inflow of fresh organic matter to soil environment and the related increase in organic carbon and total nitrogen content in afforested soils (Table 1) was certainly a significant factor that affected the increase of the activity of the analysed enzymes. The presence of carbon substrates induces and stimulates the biosynthesis of enzymes by soil microorganisms (Bielińska et al. 2014). It bears stressing that fresh organic matter not only activates the metabolic activity of microorganisms, but also has a positive effect on the rate of contaminant decomposition (Junter et al. 2002), which plays an important role in soils in industrial zones.

The abundance of easily accessible energy substances in the rhizosphere also contributes to a dynamic growth of microorganisms. During growth, roots produce organic and inorganic compounds, as well as active substances, which foster the growth of many microbes. The inflow of nutrients (root secretions, microorganism biomass) also significantly stimulates the activity of soil enzymes (Yang et al. 2005). Root secretions affect both the growth of soil microorganisms and their adaptation to the decomposition of contaminants (Bielińska and Kołodziej 2009). The stimulating effect of afforestation on the activity of the tested enzymes was particularly clear when using mycorrhized saplings (Table 2). The significant effect of mycorrhiza on the increase in the activity of enzymes catalysing the most important processes of soil transformation of organic matter, revealed during this study, is especially important under long lasting nitrogen emissions and the highly acidic pH of the soils (Table 1). Mycorrhizal fungi maintain higher metabolic activity in strongly acidic environments than other soil microbes, which is decisive for their significant participation in supplying water and nutrients to plants. Gianinazzi et al. (2005) demonstrated the positive effect of mycorrhiza on plant nutrition, tolerance to root pathogens, water deficiencies and adverse environmental conditions, as well as on the effectiveness of nitrogen assimilation by *Rhizobium*, biological diversity in the restored ecosystems, and stability of the soil environment. It is worth noting that the stimulating effect of mycorrhiza on the enzymatic activity of the tested soils is of significant practical importance to controlling processes related to the release of stored plant nutrients (Wzrost i rozwój młodników… 2015).

At the ash dumpsite, the activity of the tested soil enzymes was relatively high in the fertile soil formed 12 years ago, nearly equalling the enzymatic activity of the control soils from urban parks in Lublin and Radom (Table 2). This confirms the effectiveness of the remediation system applied to the hard coal ash dumpsite in Nowe Czarnowo that guaranties a successful development of soil formation processes and the stability of the artificially formed ecosystem.

CONCLUSIONS

1. The type and intensity of the anthropogenic pressure were factors that significantly affected the chemical properties and biological activity of the tested soils.
2. The observed high enzymatic inactivation of soils in the post-railway areas bears witness to biological degradation of the soil environment and indicates the necessity of effective remediation of such areas.
3. The wide range of enzyme activities, demonstrated in this study, proves that selected enzymatic tests may be a good indicator of both anthropogenic transformations of soils in post-industrial areas and the effectiveness of the utilised remediation system.
4. The enzymatic activity of the tested soils, shaped under diverse intensity of the anthropogenic pressure, shows that the selected biochemical parameters characterise all-encompassing phenomena of varied complexity.

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Abstract. In this study, communicative enzymatic indicators were applied to assess the quality of soils in remedied post-industrial areas. The research encompassed soils at the former railway grounds in Lublin and Radom, and soils at the ZA Puławy SA site and at the ZE Dolna Odra SA ash dumpsite in Nowe Czarnowo. The study revealed high biological inactivation in these post--railway areas. In the case of soils near the site of ZA Puławy SA and at the ash dumpsite in Nowe Czarnowo, the results obtained indicate that the utilised reclamation systems have been effective. The suggested communicative indicators, reflecting the metabolism of the soil ecosystem, enable quick assessment of the quality and health of the soils, as well as quantification of the ecological effects of the utilised remediation systems or protection programs for post-industrial area ecosystems.