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Assessment of the microbiological activity in agricultural and urban soils

Abstract: The aim of this study was to evaluate the enzymatic soil and the number of selected microorganisms in urban soil, which are located in the lane of the reconstructed road and compare it with a soil cultivated for agricultural purposes. The conducted analysis showed significant differences between the results of the soil taken from the roadway and the soil cultivated from agricultural purposes. The C:N ratio in soils of the roadway (from 24 to 31) indicated that they were degraded and heavily degraded soils. Urban soils had a neutral pH. The activity of dehydrogenase ($1.93\text{--}6.95\ \mu\text{g TPF g}^{-1}\cdot\text{h}^{-1}$), acid phosphatase ($2.42\text{--}4.92\ \text{mM pNP}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) and alkaline phosphatase ($2.34\text{--}4.80\ \text{mM pNP}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) in urban soils were low. In agricultural soils the acid phosphatase enzyme levels ranged $6.32\text{--}8.04\ \text{mM pNP}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$, and alkaline phosphatase were $7.26\text{--}9.16\ \text{mM pNP}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$. In urban soil samples collected along the roadway, a significant correlation between potassium and dehydrogenase activity, and between the C:N ratio and the activity of acid phosphatase was found.

Key words: degradation, soil, microorganisms, dehydrogenase, phosphatase

INTRODUCTION

The development of infrastructure and human industrial activity can lead to adverse changes in the soil environment and soil degradation. Depending on the mechanisms of transformation it is possible to distinguish the geomechanical, hydrological, chemical degradation and soil erosion (Mocek and Mocek-Plóćiniak 2010). During the construction and reconstruction of roads, biological changes in the soil are observed in the lane of constructed road. An important component in the soil profile is the number of different species of microorganisms which inhabit soil. In fertile and rich soils, 1 g of fresh weight of soil contains about hundreds millions of microorganisms (Szember 2001). Their abundance and activity are conditioned by many factors. The main factor affecting the development of microorganisms are the contents of organic matter (Van Veen and Paul 1981). The amount of microorganisms in soil and enzyme activity depend on such of factors as: soil pH, air-water relationships, organic matter content, C:N ratio, which are largely influenced by soil management system, including fertilization and type of fertilizer (Bielińska and Mocek 2003; Gostkowska et al. 1998). Barabasz and Voříšek (2002) argue that the most important factors influencing activity of soil microorganisms are mineral fertilization and contents of organic nitrogen.

The biological activity of the soil is evaluated mainly on the basis of the activity of four enzymes: dehydrogenases, phosphatases, urease and proteases. Dehydrogenases are enzymes which accelerate the rate of dehydrogenation reaction of given substrates in biochemical oxidation processes of organic components (Mrozowska 1999). In contrast to other soil enzymes, dehydrogenases are active only within living cells, which indicates the presence of physiologically active microorganisms in soil (Januszek 1999; Kieliszewska-Rokicka 2001). Phosphatase activity in the soil environment indicates activity of the enzymes involved in the soil colloids and humic substances (Nannipieri et al. 1990). Phosphatases are also an indicator of potential mineralization of organic phosphorus and biological activity of soil (Dick and Tabatabai 1993). According to Baath (1989), phosphatases also are correlated with an increase in the content of heavy metals in soil. Therefore, it is good for monitoring their activity in areas exposed to heavy metal pollution. Urease catalyzes the hydrolysis of urea in the soil to carbon dioxide and ammonia. They are present in many species of plants and microorganisms, especially bacteria (Januszek 1999). Proteases are catalysts in the hydrolysis of proteins in soils to simple polypeptides, spreading the peptide bonds in amino acids (Baran 2000).

The aim of the study was to determine the biological properties, activity of dehydrogenases as well as acid and alkaline phosphatases in the degraded soil taken from the lane of newly constructed roads in comparison with the same properties of soil cultivated for agricultural purposes.

MATERIALS AND METHODS

Soil samples were taken from cultivated agricultural soils (samples 1, 2, 3) and from the lane of a newly built road dedicated for use in 2013 (samples 4, 5, 6). The soil samples were collected several times during the growing season from a depth of 0–25 cm in 2013 from four locations. The samples were mixed in order to prepare one mixed sample which was tested in triplicate. The following soil properties were analyzed: the pH in 1 mol·dm⁻³ KCl (ISO 10390:1997); organic carbon (PN-ISO 14235:2003P); total nitrogen (PN-ISO 13878:2002), available forms of phosphorus (PN-R-04023:1996) and potassium (PN-R-04022:1996/Az1:2002), and available forms of magnesium (PN-R-04020:1994/Az1:2004). Two microbiological groups (bacteria and fungi) were measured. Microbiological analyzes were performed immediately after collection based on the soil of fresh homogenized samples. The total number of bacteria was determined on medium with extract of soil after 14 days of incubation at 27°C (Wallace and Lochhead 1950). Fungi were grown on Sabouraud medium with chloramphenicol after 4 days of incubation at 25°C. Testing of enzymatic activity of the soil was based on the determination of dehydrogenase activity by spectrophotometry at a wavelength of 485 nm (Thalman 1968), after 24-hour incubation at 30°C using as substrate 1% TTC (triphenyltetrazole chloride) after 24-hour incubation at 30°C using 1% TTC (triphenyltetrazole chloride) as substrate and expressed in mg TPF g⁻¹ 24 h⁻¹. Acid and alkaline phosphatase were analyzed according to procedure by Tabatabai and Bremner (1969).

Correlation between studying parameters was calculated by using Pearson's correlation factor *r* for *p* ≤ 0.05 in Statistica 10 software.

RESULTS AND DISCUSSION

It was found based on the analysis that agricultural soils were characterized by higher number of bacteria than urban soils (Table 1). More fungi were also found in agricultural soils than in urban soils located in the lane of road (Table 1). According to Kozanecka et al. (1996), apart from soil pH, also a content of organic matter and biogenic elements, temperature and method of cultivation and plant protection influence soil

TABLE 1. The number of bacteria and fungi in agricultural and urban soil

The number of sample	Bacteria [cfu·10 ⁻⁵ ·g ⁻¹]	Fungi [cfu·10 ⁻⁵ ·g ⁻¹]
1	264	188
2	180	99
3	145	108
4	88	64
5	79	63
6	98	57

microorganisms. Moreover the biological activity of the soil depends on soil type, depth of the soil profile, type of plants in cultivated soils in crop rotation sequence (Kobus 1995; Koper and Piotrowska 1996; Myśków et al. 1996).

Agricultural soils were characterized by slightly acidic reaction (pH_{KCl} 6.3–6.5) (Table 2). Despite of it the number of bacteria and fungi in agricultural soil was higher than in urban soils, which reaction was neutral (pH_{KCl} 7.3–7.9). Higher pH value in urban soils probably results from the occurrence of base rubbles, which increases the soil pH. This is a characteristic feature of urban soils (Sándor and Szabó 2014). The C:N ratio in cultivated soils was at the level of slightly degraded soils (from 16 to 19), while the urban soils of the roadway – at the level of degraded soils and heavily degraded on the (from 24 to 31) according to nomenclature by Siuta (1995). Soil microbial activity is dependent the presence of carbon and nitrogen in the soil (Januszek 1999). Dehydrogenase activity in soil profiles of agricultural soils was at the level of the soil of undisturbed biological process (6.95–14.68 mM pNP·g⁻¹·h⁻¹). In urban soils from the lane of road, this process was disrupted, therefore the dehydrogenase activity was low (0.91–1.93 mM pNP·g⁻¹·h⁻¹). Soil enzyme activity reflects the extent and degree of environmental pollution. The soils near the roadway were completely degraded and need several years to be revitalized.

In the degraded urban soils studies, in spite of a neutral reaction (Table 2), the level of the acid phosphatase activity was low (2.42–4.92 mM pNP·g⁻¹·h⁻¹). That activity of alkaline phosphatase was at a similar level (2.34–4.80 mM pNP·g⁻¹·h⁻¹). In agricultural soils the activity of acid phosphatase was at the level of 6.32–8.04 mM pNP·g⁻¹·h⁻¹, and alkaline phosphatase – 7.26–9.16 mM pNP·g⁻¹·h⁻¹. Smolik and Nowak (2003) researchers found that the enzymatic activity is an important indicator of the biological activity of the soil. According to Russell (2005), enzyme activity is a measure of productivity and fertility of soil. In

TABLE 2. Physicochemical properties of soils and activity of dehydrogenase and phosphatases in agricultural and urban soil

The number of sample	pHKCl	C	N	C:N	P ₂ O ₅	K ₂ O	Mg	Dehydrogenase	Acid phosphatase	Alkaline phosphatase
		g·kg ⁻¹			mg·kg ⁻¹			μg TPF g ⁻¹ ·h ⁻¹	mM pNP·g ⁻¹ ·h ⁻¹	
1	6.4	2.71	0.17	16	515	258	88	12.37	8.04	8.44
2	6.3	6.79	0.36	19	519	60	61	14.68	6.32	9.16
3	6.5	4.24	0.25	17	471	183	154	6.95	7.14	7.26
4	7.6	1.68	0.07	24	178	56	33	1.93	2.56	2.34
5	7.3	1.43	0.06	24	164	48	38	1.25	2.42	2.94
6	7.9	1.23	0.04	31	146	45	42	0.91	4.92	4.80

fertile soils, rich in nutrients and having regulated air-water relationships, enzymatic activity is high, depending on the amount of microorganisms in the soil. According to Mrozowska (1999), the acid phosphatase exhibits the highest activity at pH 3.4–6.2, and alkaline phosphatase – at pH 9.2–9.6. Source of phosphatases in the soil environment are mainly microorganisms, plant roots and soil fauna (Tabatabai 1994).

Statistical analysis of agricultural soil shows that there was a significant positive correlation between the content of Corg and N ($r=0.998$), between Corg and C:N ratio ($r=0.999$) at the significance level of $p<0.05$. Moreover, negative correlations between potassium, Corg and N ($r=-0.999$) was observed,

while magnesium was negatively correlated with the activity of dehydrogenases ($r=-0.999$). In urban soil located along the roadway significant correlations were determined between potassium and dehydrogenase activity ($r=0.998$), and also between the C:N ratio and acid phosphatase activity ($r=0.999$). This may indicate a positive influence of potassium contents and C:N ratio on microbiological activity. Negative significant correlations between magnesium and Corg ($r=-0.999$) with a significance level of $p<0.05$ were observed in urban soil (Table 3). Many authors (Dick and Tabatabai 1993; Myśków et al. 1996) reported, that they observed substantial dependence between the enzymes and the content of soil basic components organic matter.

TABLE 3. The coefficients of correlation between agricultural and urban soil

	pH	C	N	C:N	P ₂ O ₅	K ₂ O	Mg	DHA	PK	PAL
Agricultural soil										
pH	1.000	-0.619	-0.577	-0.655	-0.901	0.615	0.972	-0.974	0.477	-0.990
C	-0.619	1.000	0.998*	0.999*	0.217	-0.999*	-0.416	0.425	-0.986	0.504
N	-0.577	0.998*	1.000	0.995	0.165	-0.999*	-0.368	0.377	-0.993	0.458
C:N	-0.655	0.999*	0.995	1.000	0.262	-0.999*	-0.458	0.467	-0.977	0.544
P ₂ O ₅	-0.901	0.217	0.165	0.262	1.000	-0.213	-0.978	0.976	-0.048	0.952
K ₂ O	0.615	-0.999*	-0.999*	-0.999*	-0.213	1.000	0.412	-0.421	0.986	-0.500
Mg	0.972	-0.416	-0.368	-0.458	-0.978	0.412	1.000	-0.999*	0.256	-0.995
DHA	-0.974	0.425	0.377	0.467	0.976	-0.421	-0.999*	1.000	-0.265	0.996
PK	0.477	-0.986	-0.993	-0.977	-0.048	0.986	0.256	-0.265	1.000	-0.350
PAL	-0.990	0.504	0.458	0.544	0.952	-0.500	-0.995	0.996	-0.350	1.000
Urban soil										
pH	1.000	-0.444	-0.655	0.866	-0.561	-0.264	0.444	-0.327	0.890	0.725
C	-0.444	1.000	0.968	-0.832	0.991	0.982	-0.999*	0.992	-0.804	-0.939
N	-0.655	0.968	1.000	-0.945	0.993	0.902	-0.968	0.929	-0.927	-0.995
C:N	0.866	-0.832	-0.945	1.000	-0.900	-0.711	0.832	-0.756	0.999*	0.972
P ₂ O ₅	-0.561	0.991	0.993	-0.900	1.000	0.946	-0.991	0.966	-0.877	-0.977
K ₂ O	-0.264	0.982	0.902	-0.711	0.946	1.000	-0.982	0.998*	-0.675	-0.856
Mg	0.444	-0.999*	-0.968	0.832	-0.991	-0.982	1.000	-0.992	0.804	0.939
DHA	-0.327	0.992	0.929	-0.756	0.966	0.998*	-0.992	1.000	-0.722	-0.888
PK	0.890	-0.804	-0.927	0.999*	-0.877	-0.675	0.804	-0.722	1.000	0.959
PAL	0.725	-0.939	-0.995	0.972	-0.977	-0.856	0.939	-0.888	0.959	1.000

* significant correlations for $p < 0.05$; DHA – Dehydrogenase, PK – Acid phosphatase, PAL – Alkaline phosphatase.

CONCLUSIONS

1. Urban soils newly built roads are heavily degraded soil with the C:N ratio above 23. C:N ratio in cultivated soils was at the level 16 to 19.
2. Poor activity of the dehydrogenase and phosphatases in urban soils of the roadway is caused by the low contents of carbon and nitrogen.
3. The analysis of the correlation of soil degraded shows that there was a strong influence of potassium on the activity of dehydrogenases in urban soils, whereas acid phosphatase was influenced by the ratio of C:N ratio.

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Ocena aktywności mikrobiologicznej w glebach rolniczych i miejskich

Streszczenie: Celem przeprowadzonych badań była ocena aktywności enzymatycznej gleb i liczebność wybranych mikroorganizmów w profilu glebowym w pasie dróg po przebudowie. W celach porównawczych wykonano również badania gleby uprawianej rolniczo. W doświadczeniu wykorzystano zdegradowaną glebę miejską pochodzącą z terenu pasa nowo wybudowanej drogi. Przeprowadzone analizy wykazały znaczące różnice w wynikach między glebą pobraną z pasa drogowego a glebą uprawianą rolniczo. Stosunek C:N w glebach z pasa drogowego był na poziomie gleb średnio zdegradowanych i silnie zdegradowanych (24–31). Gleby miejskie posiadały odczyn obojętny. Poziom aktywności enzymów dehydrogenaz ($1.93\text{--}6.95\ \mu\text{g TPF g}^{-1}\text{h}^{-1}$), fosfatazy kwaśnej ($2.42\text{--}4.92\ \text{mM pNP}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) i zasadowej ($2.34\text{--}4.80\ \text{mM pNP}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) w tych glebach był na niskim poziomie. W glebach uprawnych poziom aktywności fosfatazy kwaśnej kształtował się na poziomie $6.32\text{--}8.04\ \text{mM pNP}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$ a fosfatazy zasadowej $7.26\text{--}9.16\ \text{mM pNP}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$. W próbkach gleb miejskich pobranych wzdłuż pasa drogowego zaobserwowano istotne korelacje pomiędzy potasem a aktywnością dehydrogenazy oraz stosunkiem C:N a aktywnością fosfatazy kwaśnej.

Słowa kluczowe: degradacja, gleba, mikroorganizmy, dehydrogenazy, fosfatazy