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DEVELOPMENT OF BIOCHEMICAL PROPERTIES IN ANTHROPIC SOIL: THE STUDY AT TŘINEC-JAHODNÁ PLOT

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Abstract

MAROSZ KAREL, VRANOVÁ VALERIE, REJŠEK KLEMENT: *Development of biochemical properties in anthropic soil (the study at Třinec–Jahodná plot).* Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 2013, LXI, No. 1, pp. 137–145

The properties of the anthropic soils formed at the sludge bed "Třinec-Jahodná". The sludge bed came from a long-time depositing of fly-ash and slag layers. Therefore, the anthropic soil properties obtained their features by both a character of layered substrate and a management of the local land reclamation. The paper presented deals with the rate of an intensity of biological and biochemical soil processes in charge of the fulfilment of plant nourishment demands, and the time viewpoints focusing on the local soil development. The set of enzymatic and biological measurements were chosen for treatments of soil bodies sampled throughout 2007–2008. The study plots inside the sludge bed and the control plot were sampled; the properties of particular horizons were studied. The results proved that the twenty-year-development of soil bodies made the proper conditions for plant nutrition. The positive statement, nevertheless, is directly linked to the presence of trees and shrubs. The vegetation seems to be one of the very crucial factors for a status of the site and maintenance of soil productivity: it affects temperature amplitudes, sensitivity to erosion, a redistribution of soil water, and a humic compounds accumulation. The statistical analyses showed significantly differing results on the study plots with a shorter development and a lower rate of vegetational cover.

soil enzymes, anthropic soils, succession, recultivation, microbial biomass

Anthropogenic soils are created by human as a result of layering substrates left from mining and industrial activities. Anthropogenic soils on the site of the Třinec-Jahodná were created as a result of a long-term layering of power–plant ash, or of irregular heaping of blast–furnace slag. Properties of such soils are therefore defined both by the characteristics of the layered substrate, and subsequently by the regulation of their further development by possible recultivations. A broad spectrum of micro-organisms and enzymes is an integral part of soil. On anthropogenic soils, with the interaction of higher plants, soil micro-organisms and enzymes are the first living component inhabiting the layered substrate, which shows primarily no biological or enzymatic activity. Nevertheless, the start–up of the processes is the key for further development of soil biota (Rejšek, 1999), for further inhabiting of sterile soils with plants and, consequently, from the point of view of the decomposition of dead organic matter, creation of humus, or degradation of toxic metals, where applicable (Markaupová, Mikanová, 2006).

By systematic monitoring of the activities of live components in newly emerging soils, ie. parameters of soil micro-organism activities, we can assess how fast the soil biota is able to inhabit and therefore start relatively natural processes in the soil. General relations of the outlined issue can be explained using a basic knowledge of this discipline. Specific knowledge of the development of biological properties or enzymatic activities in anthropogenic soils, however, opens the way to the understanding of a number of following disciplines ranging from phytoremediations, to the need for non–essentiality of recultivations and for the integration such areas into the landscape.

The aim of this paper is i) to evaluate which characteristics of biochemical properties can be used for the description of anthropogenic soils with higher amount of stress factors, ii) how fast the newly–created anthropogenic soil – *substrate* – is able to start biochemical and biological processes at the level sufficient for plant growth, and iii) how long it takes before these characteristics are comparable with natural soils.

MATERIAL AND METHODS

The site is located north of Třinec in a moderate valley near the spot Jahodná in the land registry district of Dolní and Horní Lištná, in the altitude of 400–450 m a.s.l. It is characterised by the following parameters: i) the average annual temperature 7.4 °C, ii) the average annual rainfall 984 mm, iii) the natural forest area of Podbeskydská highlands, and iv) the potential vegetation of a mixed lime-oak-hornbeam forests. More detailed characteristics of individual layers and the reference area is referred to in Tab. I.

Between 1964 and 1999, around 100.000 tons of fly–ash and slag per year was transported there hydraulically through a pipe system. Around 13 benches of different age were formed there. Due to substantial damage to the site, it is surprising that 4 specifically protected and 17 endangered or rare species of plants can be found there.

The data have been obtained from two–year systematic soil sampling and measuring specific characteristics of a complex of enzymatic activities and microbiological characteristics. The samples were collected over the years 2007–2008 of three sampling plots of each bench (the study plots) and from a comparative area. The most recent terrace had been formed 8 years before the sampling. Subsequently, the samples were processed in the laboratory at FFWT Mendel University in Brno, the Department of Geology and Soil Science. Basic descriptive characteristics of the soils from both areas have been assessed to verify comparative default parameters, which could significantly affect results, dependent, variable characteristics: the texture, pH degree (Zbíral, 2004), the parameters of sorption complex (Kappen, 1929), the content of humus (ČSN 721110, 1959), and the content of mineral nitrogen (Zbíral, 2004). Subsequently, the required biochemical and biological properties of these soils were determined: microbial biomass carbon C_{mic}, basal respiration (Zbíral, 2004), enzyme activity of acidic phosphomonoesterase, ureolytic (Zbíral, 2004) and proteolytic activity (Kandeler and Gerber, 1988).

The soil type in the comparative area is Haplic Cambisol. Anthropic soils were situated in the ash pond. The soil type of the anthropic soil at the M1 subsoil is characterised by Němeček (2001) as sandy-loamy to silty soils. The degree of sorption complex saturation has been evaluated because of the high contents of calcium, which is used for technological passages as a fully saturated (95%) soil. However, due to the absence of humus matter, clay minerals, is the total ration exchange capacity very low. The soil reaction has been evaluated as neutral to slightly

I: *Description of individual layers*

Terrace	different terrace / stand age	vegetational cover	canopy density
0	comparative plot / 30-50	*Acer platanoides, Robinia pseudoacacia, Salix* sp., *Urtica dioica, Solidago canadensis*	0.7–1
1	45 / 30	*Betula alba, Populus tremula, Robinia pseudoacacia, Epipactis helleborine, Listera ovata, Poa* sp.	0.7–1
2	41 / 30	*Betula alba, Populus tremula, Robinia pseudoacacia, Epipactis helleborine, Listera ovata, Poa* sp., *Urtica dioica*	0.7–1
3	37 / 25	*Betula alba, Populus tremula, Lupinus polyphyllus, Solidago canadensis, Epipactis helleborine, Listera ovata, Poa* sp., *Urtica dioica*	0.7–1
4	33 / 20	*Betula alba, Populus tremula, Alnus glutinosa, Lupinus polyphyllus. Epipactis helleborine, Solidago canadensis, Poa* sp.	0.7–1
5	29 / 15	*Betula alba, Populus tremula, Alnus glutinosa, Lupinus polyphyllus, Solidago canadensis, Poa* sp.	0.3–0.7
6	25 / 15	*Alnus glutinosa, Solidago canadensis, Oenothera biennis, Chamerion angustifolium, Calamagrostis epigejos*	0.3–0.7
7	21 / 10	*Picea abies, Corylus colurna*	0.3–0.7
8	17 / 10	*Corylus colurna, Swida sanguinea, Solidago canadensis, Chamerion angustifolium*	0–0.3
9	13 / 0	*Swida sanguinea, Oenothera biennis, Solidago canadensis, Chamerion angustifolium*	0–0.3
10	10 / 0	*Oenothera biennis, Solidago canadensis, Calamagrostis epigejos*	0–0.3
11, 12	8 / 0	*Calamagrostis epigejos*	0–0.3
13	8 / 0	*Ranunculus sceleratus, Typha latifolia*	0–0.3

alkaline with pH values (H_2O) 6.5–7.6. Due to evaluation of carbonates the pH decreases towards older subsoils. The content of mineral nitrogen is very low and ranges from 0.2–0.31 $\mu g.g^{-1}$ of soil. The humus content depends significantly on the age of the subsoil and varies from 11% in the oldest ones to 1% in the most recent ones. As for the assessment of contents of heavy metals, limits were exceeded for cadmium (2.23 $\mu g.g^{-1}$), zinc (181.7 $\mu g.g^{-1}$) and lead (154.8 $\mu g.g^{-1}$), whereas nickel and copper values are standard (37.7 or 64.2 $\mu g.g^{-1}$, respectively).

The Cambisol is characterized by the features of grain–size composition as aluminum to sand–clay. The level of sorption complex level has been assessed as saturated (75 %). The soil reaction has been assessed as slightly acidic with pH (H_2O) 5.8. The content of mineral nitrogen is very low and ranges from 0.2–0.25 μg/g of soil. In terms of content of humus, the soil is characterized as humic, the content of humified organic matter reaches 7.5%. In this soil unit no values of heavy metals content have been exceeded.

Regarding the default properties of compared areas it can be noted that, with the exception of heavy metals contents, no major differences in the observed parameters have been noted in these soil units. Even though these are two different soil units, in respect of their fundamental characteristics (texture, pH, sorption complex parameters, content of humus) they are very similar. The Haplic Cambisol was therefore selected as appropriate for the comparison with substrates in the soil pond. For the assessment of the hypothesis – how quickly a substrate is able to reach such biochemical indicators, in which plants will not be significantly stressed by the insufficient activity of this component – all values of observed parameters have been set up to the level, when they move from the assessment criteria "low" to the assessment criteria "medium". These criteria of assessment stem from a long-term study of enzymatic activities on a wide scale of soils and localities of various ecological characteristics.

For assessment of the second hypothesis – after what time the assessed biochemical characteristics come close to the level in the comparative area – statistical significance level p = 0.1 was chosen and the statistically significant differences between the comparative area and the individual benches were assessed by the STATISTICA program and the Fisher LSD test. In the graphs vertical columns mark 0,9 confident intervals.

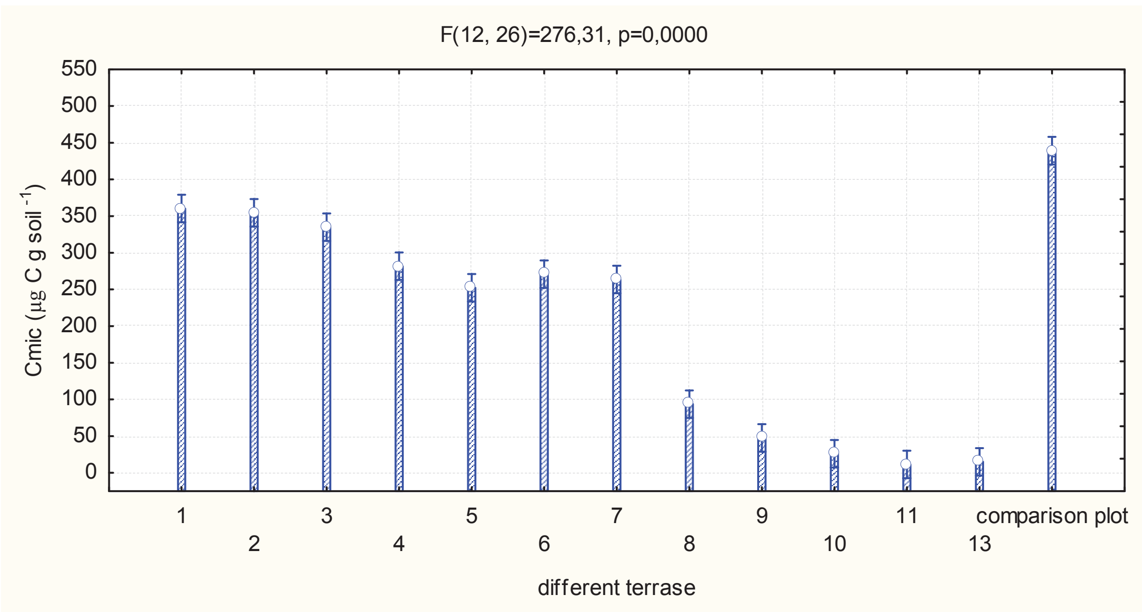
RESULTS AND DISCUSSION

In this chapter, the results of found biochemical properties are presented individually so that the differences between the benches of mud ponds and the comparative area are clearly visible for each defined soil property.

Carbon of microbial biomass C_{mic}

Soil micro-organisms are an important component of the substance cycle between the plant and soil, they support the growth and development of plants, and they also contribute to the availability of nutrients for plants. They can therefore be used as the appropriate indicator of soil quality. In conclusion, soils with a high microbial component are more suited to satisfy the needs of plants than soils with limited activities of this component. For soils showing a very limited supply of nutrients, micro-organisms become all the more important supply of nutrients.

The presented results clearly show that the amount of microbial biomass can be assessed both



1: *Microbial biomass carbon in soils different terrace and comparative plot* in the comparative area and at benches of more than 20 years as high or very high. However, practically none of the benches is close in a statistically significant manner to the comparative area. The exception is 2 of the oldest benches with plant cover of approx. 30 years, where leaf fall from tree species has had a positive impact, and, subsoil has developed with a higher content of organic matter (Fig. 1). Other authors (Lencová, 2009) describe, in similar condition values, C_{mic} most frequently in the range of 250–450 µg $C.g^{-1}$. For anthropic soils, Vácha et al. (2005) stated 127.9–260 µg $C_{mic}.g^{-1}$. For agricultural soils, Hábová (2012) presented 93–140 µg $C_{mic}.g^{-1}$.

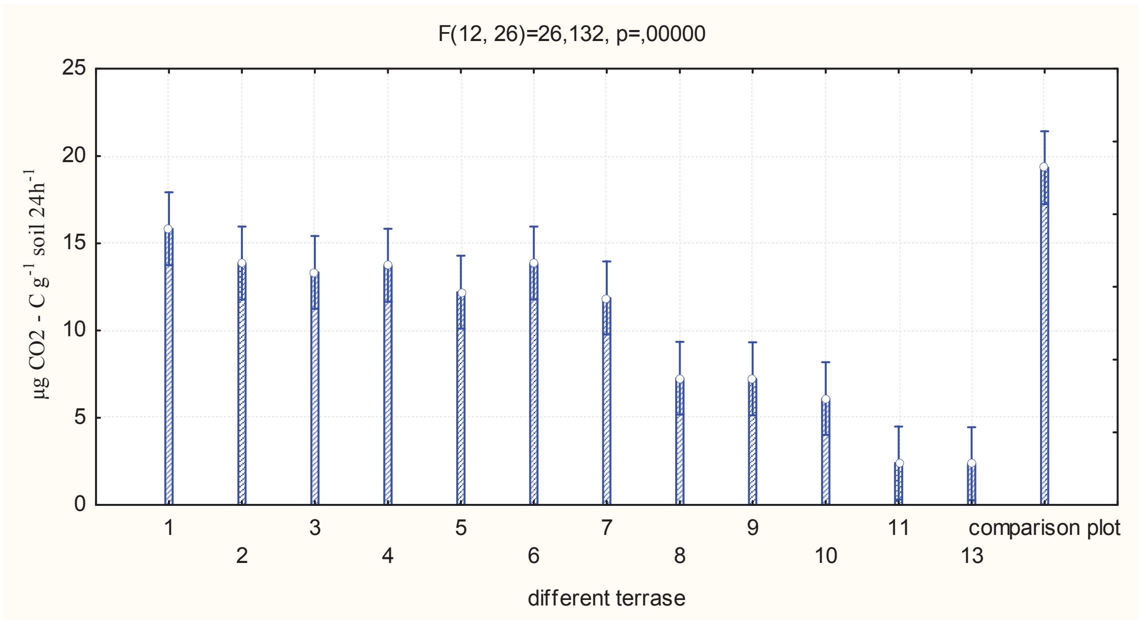
Basal soil respiration

Soil respiration activity is the most widely used measure of the soil microflora activity. It corresponds to the quantity of CO_2 produced by soil microflora within a specified time. Respiratory activity of the soil is a general indicator of mineralisation capacity of microbial settlements, and expresses mineralisation of organic matter and is used as an index of soil fertility.

The results display that the rate of respiration in the comparative area is assessed as moderate and in benches less than 20 years it is assessed as low. Level, when the observed parameters of basal respiration, reach higher values is achieved in benches older than 20 years (Fig. 2). Hendrix *et al.* (1986), Raus (1999) describe very similar values of basal respirations in natural soils: between 7–15 µg $CO_2.g^{-1}$ of soil. $24h^{-1}$. It is likely that the rate of respirations is positively affected by at least a gappy shading of tree species. However, at statistical evaluation of individual benches, virtually none of them shows values similar to those of the comparative area. Only the oldest bench No. 1 comes close to this level, similarly to bench 6 with a gappy coverage of alder which, due to its leaf dropping with favorable chemical composition (higher content of N) can contribute, as a substrate, to the increase in the respiration activities, and to increase the quantities of active microbial biomass. Similar conclusions have been noted by Hendrix *et al.* (1986), argues that the values of basal respiration correspond to differences in bacterial biomass. Selected kinetic parameters of soil microbial respiration were reported by Vranová *et al.* (2009). As a result of anthropic soil respirometry analyses Vácha *et al.* (2005) stated 1.3–12.24 µg $CO_2.g^{-1}$. $24h^{-1}$.

Enzymatic activity

Soil enzymes come from a number of different sources, including microorganisms, plants, animal excrement (urine and faeces), decomposition, dry and wet deposition (including leaching from vegetation, throughfall and stemflow) and from enzymatic fertilizers used to improve crop nutrition. Soil microorganisms produce enzymes to recycle soil organic matter, thus ensuring microbial nutrition, they are important for the nutrients cycle in the soil, for humus-creation processes, plant nutrition and maintaining ecological balance. Characteristic activities of soil enzymes can be used for the study of biochemical processes in the soil, for exploring the microbial ecology and they also indicate the soil quality. In this way the level of damage or naturalness of the soil environment can be assessed. Enzymatic activities as an indicator of quality of the soil have several advantages and disadvantages: they are in close relation to important soil characteristics, they may show changes before other soil properties do (Dick *et al.*, 1996), but their



2: *Basal soil respiration in soils different terrace and comparative plot* specific sensitivity to some stress factors or default properties of soils may provide data of limited applicability.

Proteolytic activity

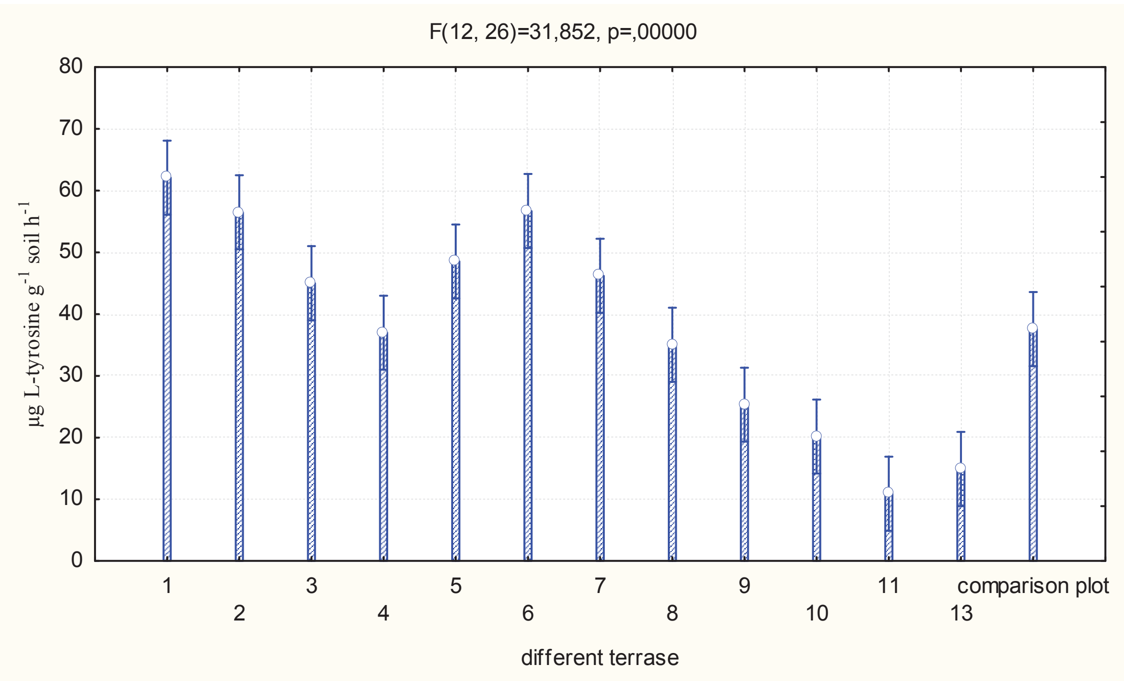
Proteases are composed of a complex of proteolytic enzymes which come from roots of plants, dead tissue, faeces, but most frequently from activity of microorganisms. Production of proteases by micro-organisms is highly dependent on environmental conditions. If the soil becomes stressed and the living conditions of micro-organisms get worse, the production of this enzyme is increased. Proteases play an important role in transformations of nitrogen substances in the soil. Proteolysis is an important process in N–cycling in many ecosystems, as it is considered to be a rate–limiting step of N mineralization in soils (Weintraub and Schimel, 2005). Proteolytic rates commonly change through the seasons (Watanabe and Hayano, 1995; Moscateli *et al.*, 2005), and do not always correlate with total numbers of soil bacteria or NH_4^+ and NO_3^- availability. Protease activity, like any other enzyme activity, depends on soil type (Geisseler and Horwath, 2008) and decreases with soil depth due to a decrease of organic matter content and microbial abundance (Bausenwein *et al.*, 2008; Madejón *et al.*, 2009).

From the results of proteolytic activities it is clear that its values vary, in fact independent of the age of the benches. However, like other enzymes, proteases are limited by their environment. Where there are significant temperatures and humidity fluctuations (benches with thin vegetation) the values of proteolytic activities are very low. In benches with at least partially developed plant coverage, they show significantly higher values.

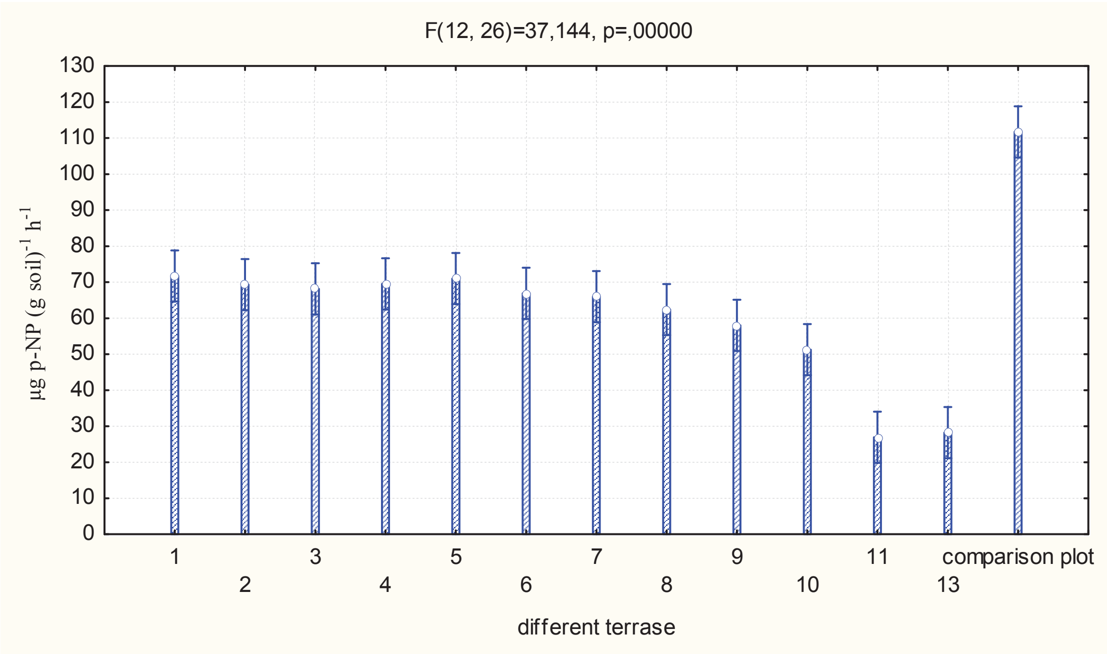
If we take into consideration i) the presence of toxic metals, ii) vulnerability of fly-ashes substrate to drying–out, and iii) lack of colloidal fractions, on the basis of the claims that the activity of proteases increases with increasing stress of micro-organisms, the following hypothesis can be made: proteolytic activity significantly exceeds the values noted in natural soils due to important stress factors. But even these stressful factors have a limit and when excessing the limits in the most recent benches even proteolytic activity is reduced to a minimum (Fig. 3). Lipson *et al.* (1999), note proteolytic activities in the wide intervals 20–170 μg L–tyrosine g^{-1} soil h^{-1} soil in the conditions in natural soils. According to Kandeler *et al.* (1999) casein protease activity in forest soil up to ca. 3600 μg L–tyrosine g^{-1} soil h^{-1} in H horizon and Enowashu et al. (2009) found casein protease activity in a spruce stand up to ca. 1 700 μg L–tyrosine g^{-1} soil h^{-1} in a O, A and B horizon (substrate casein, incubation temperature 50 °C). Compared to them García–Gil *et al.* (2000) reported casein protease activity in soil treated by municipal solid waste soil depth 0–20 cm up to 7 μg L–tyrosine g^{-1} soil h^{-1} (substrate BBA, incubation temperature 39 °C).

Activity of acid phosphomonoesterase

Acid phosphomonoesterase location in soils was reviewed by Rejšek *et al.* (2012). Phosphomonoesterases are important enzymes involved in the P cycle of soil and aquatic environments. Acid phosphomonoesterase (APM) is produced in rhizosphere and bulk soil by various



3: *Proteolytic activity in soils different terrace and compative plot*

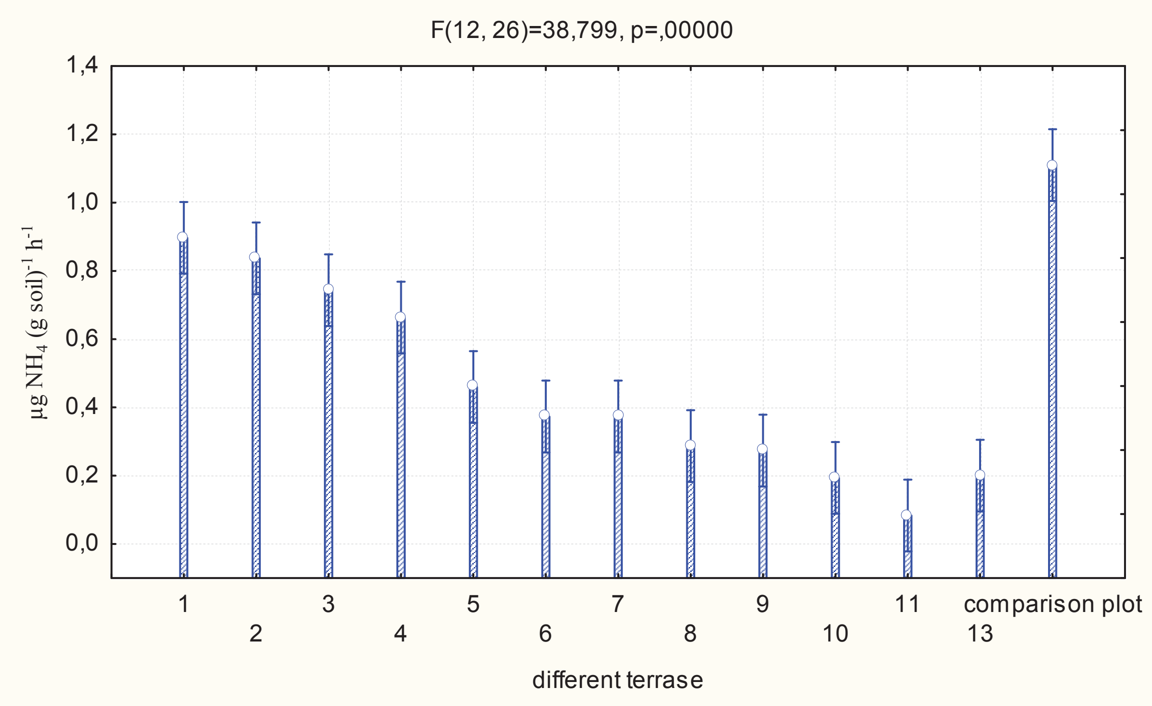


4: *Activity of acid phosphomonoesterase in soils different terrace and comparative plot*

living organisms including bacteria, protozoa, mycorrhizal fungi, and saprophytic fungi, as well as in the form of plant–root exudates (Rejšek, 1991; Asmar and Gissel–Nielsen, 1997; George *et al.*, 2008; Rejšek *et al.*, 2012). They are also transferred to the soil environment along with soil micro-organisms and roots of higher plants. Activity of phosphates favorably influences the quantity of organic matter in soil, humidity and the quantity of nitrogen. Degradation and mineralisation of organic matter in soil is the basis for the uptake of phosphorus by plants. If there is enough available phosphorus in soil, the activity of phosphates is reduced. Acid phosphomonoesterase in soil is affected by many factors and soil properties. Although no correlations were found between organic–P content within the range of tested soils and their APM activity, positive correlation between APM and total P content in these soils. Negative correlations were found between APM activity and the quality of humus, available P, pH, and clay content (Šarapatka and Kršková, 1997).

In a comparison Klose and Tabatabai (2002) reported a weak correlation between APM activity or protein concentrations of APM in soil and soil pH.

The results show that the activity of phosphatase is evaluated on all artificially created benches as very low. This also partly follows from the fact that acid phosphomonoesterase have their optimum in acid soils with a pH lower than 6 and it therefore cannot be expected that in neutral and slightly alkaline soils – their ecological optimum is not existent here – they would show a high value (Fig. 4). Other authors (Šarapatka, Kršková, 1997; Formánek *et al.*, 2006) indicate around 10–30 times higher activity of phosphatases in the soils with slightly acidic pH. In respect of the enzymatic activity, another inhibiting factor becomes significant: toxic pollution. In accordance with Mikanová *et al.* (2006), a significant inhibition of activities at contaminated sites was observed in all enzymes. The exception is the activity of the enzymes which are directly dependent on the content of a particular element in the soil (arylsulphatase – sulphur, phosphatase – phosphorus, urease – nitrogen), which are related to the cycle of the element in the soil and therefore in this case it does not indicate the degree of damage to the soil microflora, but it is dependent only on the content of the element in the soil. The important role in making available phosphorus from organic matter is played by neutral and alkaline phosphatase. For the application of this method of determining, in relation to the pH in substrates and the correlation area, we must argue that most probably the values of enzymatic activities between comparative area and benches will most probably never be equal because of different values of soil reaction and the associated optimum for the activation of acidic phosphomonoesterases. Acid phosphomonoesterases activity was reported in the range from 0 to 104 µmol. g soil^{-1}.h^{-1} (Rejšek *et al.*, 2012) and fluctuates throughout the growing season (Boerner *et al.*, 2005; Formánek *et al.*, 2006). Extraction of acid phosphomonoesterase from soil was disscused by Holík *et al.* (2011) and Rejšek *et al.* (2012), and proportion of root–derived acid phosphomonoesterase in total soil acid phosphomonoesterase in different forests was reported by Dundek *et al.* (2011).



5: *Ureolytic activity in soils different terrace and comparative plot*

Ureolytic activity

This activity is in close relation to the nitrogen cycle. The urease activity means the decomposition of organic nitrogen substances in the soil. In the soil environment it is involved in the hydrolysis of urea into NH_3 and CO_2 and therefore it makes nitrogen available for plants. This is a very stable soil enzyme, which is used in the assessment of soil fertility. Ureases are sensitive to contamination by foreign substances.

On the basis of results achieved, we can argue that the ureases activity in all observed areas is very low (Fig. 5). Mikanová, Kubát (2005) has noted 5×–10× higher value of ureolytic activities in naturally incurred soils, but with an increased content of toxic metals. As well as Bremner and Mulvaney (1978) and other state around 10 times higher values in natural soils. Referring to the fact that ureolytic activity is significantly dependent on the content of mineral nitrogen in the soil, the results reinforce the findings. A steep decline is obvious depending on the content of NH_4 and NO_3 towards the most recent benches was presented. It can therefore be argued that ureolytic activity directly depends on the quality of vegetation cover and the humus content. But even in the oldest benches the same values are not achieved as in comparative areas and it can be argued that as a result of a lack of any nitrogen in the soil, these anthropogenic substrates did not manage to reach more favourable values within the activity of urease after the 45 years of age.

CONCLUSIONS

The study area was made of at around 100.000 tons of fly-ash and slag accumulated within one year during the period 1964 and 1999. The area was composed of around 13 benches of different age covered by varied vegetational communities. The investigations were aimed in the evaluation of soil biochemical parameters related to the age of the benches, the role of comparative plot with a natural soil development was concerned with. The evaluation was based on the standard limits of high/low activities focused on an estimation of time when the study benches made on fly-ash display the established vegetational communities.

The paper, in addition, was on the subject of a methodological verification of routinely used laboratory assessments for investigations of the kind. We prove an ambiguous significance of phosphatase activity measurements (due to soil pH degree) and urease activity measurements (due to a high heavy metal contents).

The statistical assessments, carried out under the $p = 0.1$, led the authors for a confirmation of minimally a twenty-year-development of such bench for reaching the established vegetational communities. The interrelationships among the age of benches and soil biochemical parameters were described where a role of relations between a vegetational community and soil biochemical parameters were highlighted only on the general level.

The data presented support the approach when the influence of stress factors, such as significant temperature differences in the dark soil surface in the course of the day and night, together with drying out of upper horizons and finally, the presence of initial humus subsoil, which also helps buffer other inhibiting factors (the weak water-holding capacity, the absence soil colloids, the content essential nutrients) is eliminated. This argument is supported by other authors, as well. According to Moravec and Jeník (1994) fytocenoses are influenced by the soil; however, they affect the soil themselves: this process is autoregulative and its direction, which is irreversible, is determined in particular by the local climate, a parent material and a relief of the area. Forest coverage (E3) including the bush (E2), herbal (E1) and moss (Eo) layer affects the condition and characteristics of forest soil mainly by the quantity and quality of its dead tissue, by the influence of humidity and temperature conditions of the site, the protection of forest soil against water erosion and deflation.
On the basis of statistical evaluation it can be noted that benches with the full canopy closure and with a higher content of organic matter in upper subsoils can relatively soon come close in the monitored criteria to the subsoils of naturally incurred soil, where the characteristics of these parameters are given by a long-term natural development. Authors assumed that a natural succession processes would ensure reaching certain level of development of soil characteristics within approx. three decades, whereas artificial intervention can significantly shorten the required time. These interventions, however, will deprive us of the valuable chance to monitor change and development of man–made substrates in *semi–natural* functioning society.

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