

## DEVELOPMENT AND APPLICATION OF ECOLOGICALLY BASED INDICATORS OF SOIL QUALITY

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Soil quality represents an integral value of the compositional structures and functions of soil in relation to its use and outer conditions. Undoubtedly, soil organisms, including microorganisms, belong to the basic components of the soil environment and play a key role in different ecologically important soil functions. This is true especially for the maintaining of matter and energy transfer in terrestrial ecosystems. Under stress conditions caused by chemical pollutants or other anthropogenical effects, the development of different soil microorganisms and their biochemical activities usually undergo alterations. In order to indicate beforehand possible negative ecological consequences of anthropogenical disturbances to soil functions, microbiological and biochemical parameters, should be considered. In our respective approach to assessing soil quality by ecologically related biological parameters, this assumption was tested in an international project by scientists from five European countries. After evaluation of more than twenty parameters applied to samples representing 49 different soil sites, it has been concluded, that N<sub>2</sub>-fixing bacteria, total microbial biomass, dehydrogenase activity, nitrification/denitrification, soil respiration (CO<sub>2</sub> release), and a humification capability of soil microorganisms might represent useful indicators.

soil quality; soil ecology; microbiological and biochemical indicators

### INTRODUCTION

All living organisms modify directly or indirectly their environment and this is true also for humans. Human alteration of Earth's ecosystem based on agriculture, industry, recreation and commercial activities become a substantial factor for land transformation with consequences for a global environmental change (Vitousek et al., 1997b). This change may include alteration of soil quality and its attributes such as soil productivity, human



and animal health, and environmental quality. In our understanding, soil quality is an integral value of the compositional structures and functions of soil in relation to its use and outer conditions. Because of its complexity, heterogeneity and differences in use soil may undergo alterations at different scales. For this reason, it is rather difficult to make an evaluation of soil quality in a simple way. From the agricultural point of view, soil fertility served as a reliable indicator of soil quality for decades, and it developed mainly positively. *Mats on et al.* (1997) demonstrated this by pointing on an average increase in yield of wheat by 100%, and of corn up to 500% from 1940 until 1990 in Colorado. However, such a criterion is not environmentally adequate, for it does not reflect hazards to soil and its functions caused, e.g., by the extensive use of chemicals in agriculture. Similarly, the density of industrial emissions and other adverse human activities might not be reflexed using soil fertility as a parameter of soil quality. A threat to soil environmental functions has been recognized long ago (*Filip, 1973; Kovda, 1975*), but the believe in natural self-purification capacity of soils persisted for long (*Möller, 1983*).

The first moves towards the development of soil protection in Europe were made in Germany and the Netherland, and later in the European Community (*Barth, L'Heremite, 1987; Howard, 1993; Thormann, 1984*). Since then, the necessity to evaluate physical, chemical and biological soil characteristics as a prerequisite of an effective assessment of soil quality has been stressed repeatedly, and different physical and chemical parameters have been already adopted internationally (*Arshad, Coen, 1992; Karlen, Stott, 1994*). However, biological parameters remained a matter of discussion until yet. This is mainly focussed on consequences for soil fertility, water and air quality and on some sanitary aspects (*Chaussod, 1996*). Several attempts were made in a general way to indicate the usefulness of biological methods in evaluation of soil health (*Linden et al., 1994; Torstenson, 1997; Turco et al., 1994; Visser, Parkinson, 1992*), but an ecologically based conceptual approach has not been developed yet. Instead, numerous individual approaches were presented (see in 16th Vortragstagung, 1996) which, however, have only a little chance to contribute to the soil protection practice and policy. Rather, they might evoke some doubts among the environmental policy makers as to the practicability of the biologically based parameters and perhaps as to their need at all in the soil quality assessment. Therefore, in this paper, we attempt (i) to underline the necessity of a process-linked biological approach in the assessing of soil quality, (ii) to indicate the usefulness of some ecologically based parameters, and (iii) in this way to contribute to the development and international harmonization of methods in the field of soil protection science, practice, and policy.

### Effects of soil organisms on the global environment

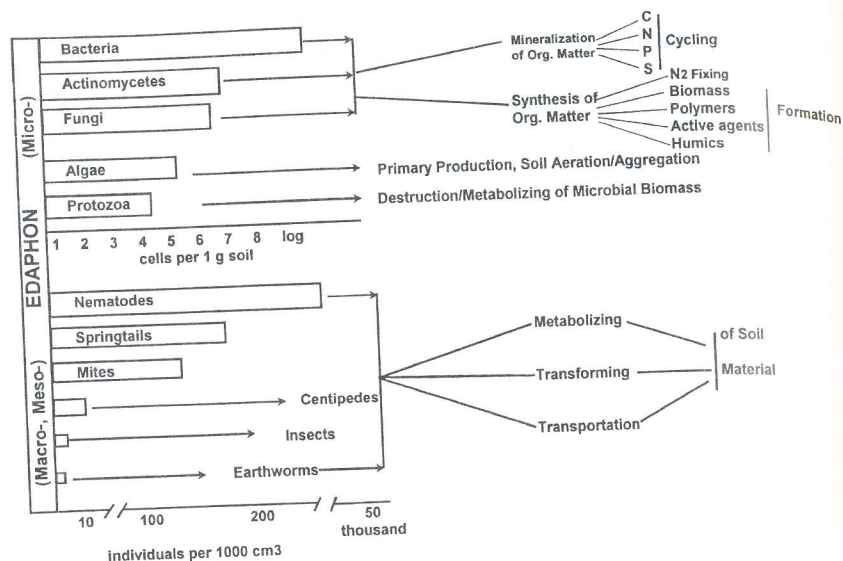
Among terrestrial ecosystems, soils contain by far the highest numbers and the greatest diversity of organisms. In a trivial comparison, there are at least twice as much or more microorganisms in a teaspoon of soil than the total population of humans on the Earth. These organisms, i.e., edaphon, do not only inhabit soils but they also actively contribute to the transformation of their own natural habitats. In a comparison of the time scales for biological and physical processes involved in the development of terrestrial ecosystems, the former take only 1–100 years, while for the latter usually > 100–10 000 years should be calculated (*Dobson et al., 1997*).

The approximate numbers and the main functions of different parts of edaphon in arable soils are schematically shown in Fig. 1. Bacteria (including actinomycetes) and fungi represent the most active groups of soil organisms. They are involved in both degradative and agradative biogeochemical processes at a given site. The decomposer community including organisms capable of degrading lignin, cellulose, pectin, chitin, protein and other organic compounds contribute to the integrity of the ecosystem by converting different remnants of plant animal and microbial tissues. Via mineralization of organic matter and a partial immobilization of the released nutrients, soil microorganisms maintain the predominant part of the matter and energy transfer in terrestrial ecosystems.

Since the life on Earth is based upon carbon, CO<sub>2</sub> is the main final product of microbial mineralization of organic compounds. Soil represents a major source in liberating CO<sub>2</sub> to the atmosphere. Under steady state conditions in an ecosystem, the CO<sub>2</sub> emission should roughly equal the respective demand of photosynthetically active plants. However, long-term measurements combined with an interpolation of data available from the past indicate that due to different activities, humanity added CO<sub>2</sub> to the atmosphere resulting in a concentration increase of about 30% relative to the pre-industrial era. Some scientists believe that this increase has been driven primarily by fossil fuel combustion (*Vitousek et al., 1997b*); others claim that non less than two-thirds of the increased atmospheric CO<sub>2</sub> may come from an increase in soil respiration (*Bertram, 1991*). Usually, about 74% of soil CO<sub>2</sub> is related to microbial activity and 26% to a root respiration which, however, strongly depends on the activity of rhizospheric microorganisms inhabiting plant roots (*Titljanova, Tesarova, 1991*). Consequently, the community of soil microorganisms-decomposers and its mineralization activity expressed in CO<sub>2</sub> release, should be considered as an important parameter characterizing soil quality from the ecological point of view.

Data also exist that up to 50% of plant residues in soil could be digested by invertebrates (*Pustian et al., 1990*). However, since those soil animals





1. Approximate counts and ecologically important activities of soil organisms

are mainly capable only of an uncomplete utilizing of plant polysaccharides, their contribution to the mineralization of organic matter in soil can be rather neglected (Curry, Good, 1992).

From the energetical point of view, mineralization of organic matter represents a catabolic process releasing energy for anabolic (synthetizing) activities of soil organisms. A close relation may exist between anabolic activities of soil microorganisms and the global carbon cycling. Norby (1997) calculated, that only about 45% of the globally increased CO<sub>2</sub> amounts remains in the atmosphere while the main part is apparently missing. That missing carbon might be assigned to soil environments. In that case, CO<sub>2</sub> could be transferred below ground either into a labile, short-lived pool such as microbial biomass, or into a refractory soil organic matter such as humic substances. Thus, the assessment of anabolic activity of soil microorganisms, i.e., the estimation of carbon balance between soil microbial biomass, humic substances and CO<sub>2</sub> release would contribute both to the elucidation of global carbon budget and soil quality from the ecological point of view.

Among the primary greenhouse gases involved in the global cycling of carbon, the warming potential of methane is much higher relative to CO<sub>2</sub>, and some 84% of CH<sub>4</sub> originate from biotic sources (Dale, 1997). Those

sources, however, usually do not include natural soils with exception of wetlands. Therefore, CH<sub>4</sub> emission apparently would not belong to primary criterions of soil quality, but rather to those of soil use and management, especially for paddy soils.

Among the major elements required for all forms of life, nitrogen is unique in that it exists in vast amounts in the atmosphere. From that reservoir, N<sub>2</sub> must be bound into a living matter by the activity of free-living and/or symbiotic microorganisms mainly in soils and also in waters. Although human activities may add as much fixed N<sub>2</sub> to terrestrial ecosystems as do natural sources (Vitousek et al., 1997a) for the enormous importance of bacterial N<sub>2</sub>-fixing activity in the global N cycle, this process should not fail in the assessment of soil quality.

Beside the N<sub>2</sub>-fixation, also other key processes of the N cycle, such as ammonification of nitrogen bound in organic compounds, an oxidation of NH<sub>4</sub><sup>+</sup>-N to NO<sub>3</sub><sup>-</sup>-N (nitrification), and a reduction of NO<sub>3</sub><sup>-</sup>/NO<sub>2</sub><sup>-</sup> to N<sub>2</sub> (denitrification), represent microbially mediated soil processes which may be useful in a system of ecologically based parameters of soil quality. This is because an anthropogenical alteration of primary processes in the cycle of nitrogen may have multiple consequences, such as (i) an increased concentration of the greenhouse gas N<sub>2</sub>O and/or other No<sub>x</sub> gases globally, (ii) losses in soil nutrient, (iii) acidification of soils, and (iv) increased transfer of N from soil to aquatic environments (Vitousek et al., 1997a). For the emission of NO<sub>2</sub> soils have been identified as being by far the largest source (Bani, 1986).

Not only the cycles of carbon and nitrogen may become strongly affected by human activities. Similar is true also for the S, P and other elemental cycles. However, the key roles of C and N in the biosphere, the important role of microbially mediated processes in the transformation of these elements, and also a variety of analytical methods available, may account for the priority involvement of C and N transformations in the biological assessment of soil quality.

An anthropogenically based pressure on soil organisms and their activities may cause a threat to biodiversity in soil environments. Although there is no sufficient knowledge available yet on the dependence of the most of soil processes on a degree of biodiversity in a soil environment, and no reliable methods exist to control this parameter in soil habitats, the preservation of biodiversity should be generally attempted with respect to soil quality conservation. As a general rule, higher diversity would mean longer food-chains, more cases of symbiosis and greater possibilities for negative feedback control, which could reduce oscillations and hence increase stability of an ecosystem (Bianchi, Bianchi, 1995).

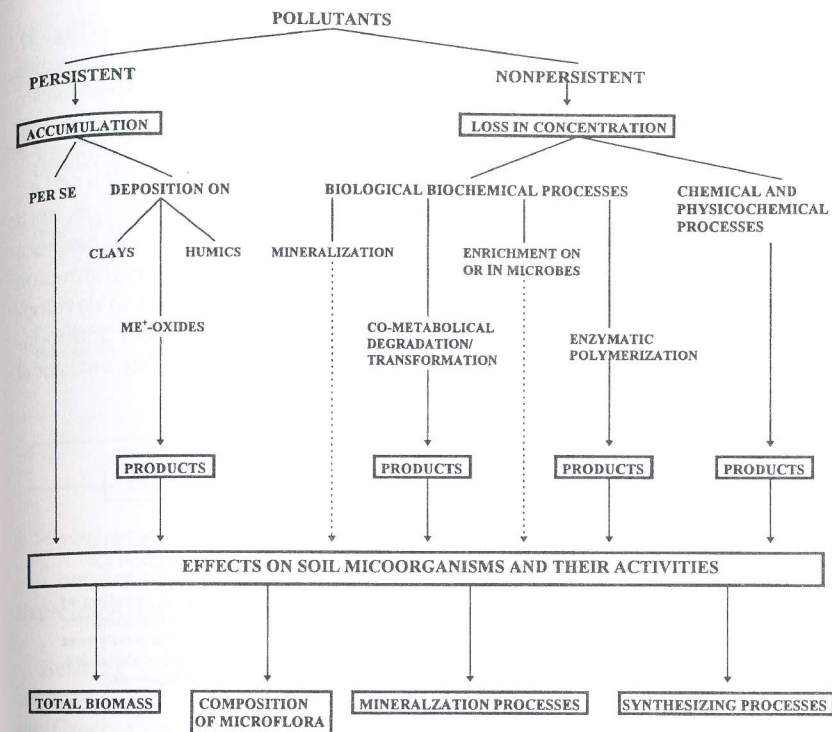


## Effects of anthropogenic pollutants on soil microorganisms and their activities

Soil comprises a structurally complex mosaic of heterogeneous microsites colonized mainly by microorganisms. In a biologically less active podzolic soil, e.g., up to  $9 \text{ t ha}^{-1}$  (d.w) of bacteria and about the same amount of fungal biomass may exist in a plough layer (Nikitin, Kunc, 1988). From an average size of a bacterial cell, a total surface of about 500 ha bacteria per ha of soil can be postulated (Kas, 1966). These numbers and the capability of soil microbes to multiply even under undesirable environmental conditions relatively quickly, signalize the high degree of susceptibility of soil microorganisms against both positive and/or negative outer effects, e.g., those caused by pollutants. Different ways exist on how anthropogenic pollutants may affect soil microorganisms (Fig. 2). In general, either directly or via degradation or transformation products, pollutants can affect the total microbial biomass, individual groups of microorganisms, and also different microbially mediated processes (Filip, 1995). Because of their ecological importance, all these individual targets should be considered as potentially useful parameters in assessing soil quality.

### Methodological approaches in biologically based indication of soil quality

In their comprehensive work on applied ecotoxicology, Römcke and Moltmann (1996) summarized the major effect tests actually used for terrestrial media. Those are mainly tests with higher vertebrates (birds, mammals), soil saprophagous invertebrates, plants and (for pesticide side effects) pollinators, such as honey bees. The authors pointed on the existing lack in soil tests with microorganisms. Graefe (1997) presented some soil biological diagnostics based purely on analysis, classification and evaluation of soil invertebrates. He diversified among organisms capable of indicating soil freshness, wetness, acidity, alkalinity, etc. Yakovlev (1997) recommended the abundance of selected protozoa (*Amoebae*, *Colpodida*) and algae (*Heterotrix* sp.) in soil samples or their behavior in a soil suspension for the characterization of virgin and anthropogenically affected soils. Debus and Hund (1997) prepared of soil extracts and recommended using aquatic test organisms in their strategy for the assessment of soil contaminants, although in their previous work, aquatic tests with *Daphnia magna* and *Scenedesmus subspicatus* appeared rather less sensitive (Hund, 1994). The same authors also performed tests with natural soil samples, but only in order to target for organisms of different trophic levels such as individual microorganisms, algae, nematodes and higher plants (Debus, Hund, 1997). In our opinion, all those assessment strategies might have some advantage of simplicity and the methods used might be perhaps easy to standardize. How-



2. Behavior of pollutants in soil and their effects on microorganisms

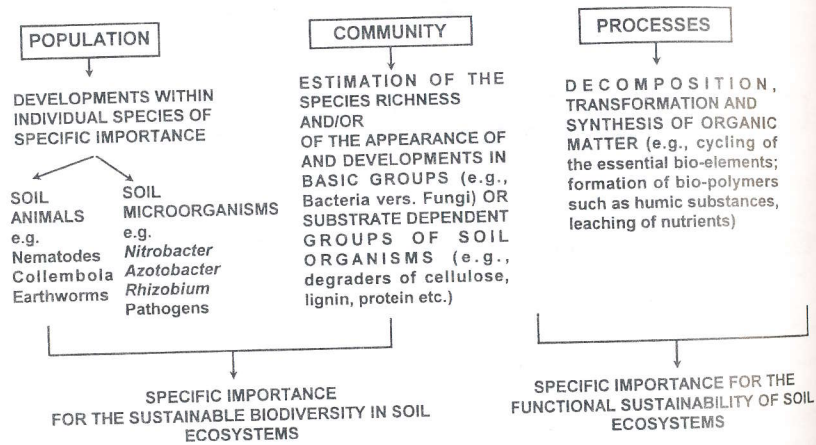
ever, all those strategies oversimplify soil both in its biological and abiotic structural complexity and completely disregard soil ecological functions. Thus, they should be only used for toxicity testing of individual pollutants entering the soil environment or for estimating the fate of pollutants in food-webs which include soil biota.

It might be also of interest to consider fungal-plant symbiosis such as ecto- and endo-mycorrhiza as indicators of soil quality. Such associations exist by more than 90% of the plant roots, and the degree if symbiosis has been found to integrate different environmental parameters (Domsch, 1985; Weisenhorn et al., 1995a, b).

In an ecologically based approach of soil quality assessment, a population level should be undoubtedly involved but only for organisms of a recognized importance for the sustainable biodiversity in soil or those playing a key role

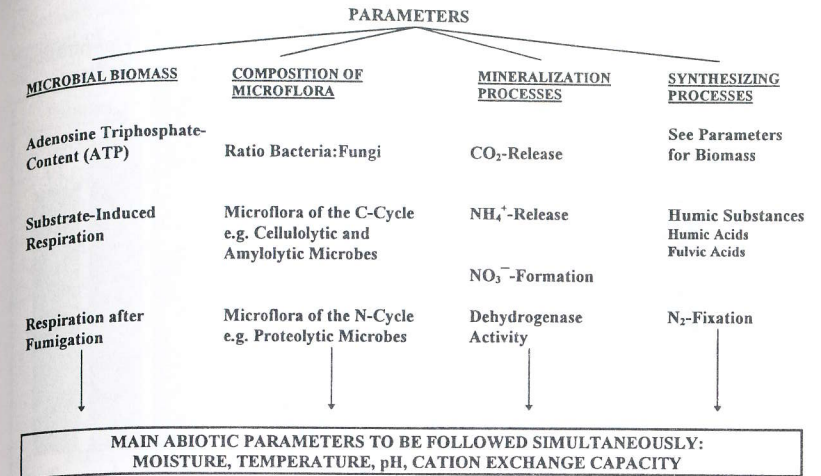


in the ecologically important processes, e.g., in the nitrogen fixation (Fig. 3). Certainly, with several millions species of invertebrates in soil, and thousands species of bacteria in every gram of a soil sample, it is almost impossible to make a survey on biodiversity with currently available methods. In addition, it is unclear until yet, as whether there is a correlation between soil ecological functions and the degree of biodiversity in soil organisms (Wardle, Giller, 1996). Contrary to this, there is no doubt that firm linkages exist between different microbial communities, their activities, and between ecologically important processes, such as mineralization and transformation of organic matter (Figs. 1, 3). These facts can open several ways to developing assessment and evaluation of soil quality in both natural and anthropogenically stressed soil sites by employing several already available analytical methods.



3. Characteristics of population, community and process related levels in the bio-indication of soil quality

Based on these presumptions, we preselected some prospective soil biological parameters as shown in Fig. 4. These include the estimation of microbial biomass, composition of microbial communities, mineralization processes and synthesizing processes. Simultaneously, the basic physico-chemical soil characteristics have to be estimated. To make such system feasible for application, internationally adopted and standardized methods should be applied as far as possible. Otherwise, novel methods and other improvements could be easily adopted.



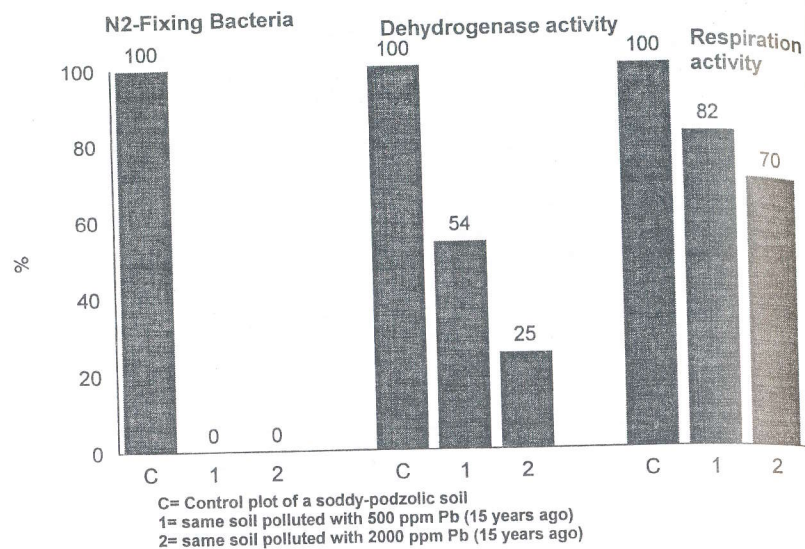
4. Selected parameters to assess ecologically important soil characteristics

#### Experience from an international research project and some open questions

Between 1995 and 1997 our approach to assessing soil quality by biological and biochemical methods was tested by five groups of soil biologists from the Czech Republic (headed by J. Kubat, Prague, and M. Tesarova, Brno), Hungary (headed by T. Szili-Kovacs, Budapest), Russia (headed by D.G. Zvyagintsev and M.M. Umarov, Moscow), and Slovakia (headed by P. Bielek, Bratislava). The international investigations were co-ordinated by the senior author. Soil samples from 49 sites representing different European soil types, and which were differently anthropogenically affected, were collected several times and analyzed according to the scheme shown in Fig. 4. All analyses were performed in laboratory because under field conditions natural stresses such as variations in temperature and moisture sometimes could fully mask low effects of anthropogenically caused soil alterations. All teams used the same analytical methods for their experiments which were based either on the available DIN/ISO standard methods or on the ASA methods of soil analyses (Page et al. 1982).

Fig. 5 shows the high sensitivity of methods such as N<sub>2</sub>-fixing bacteria, dehydrogenase activity, and respiration activity against a long-term pollution of a soddy podzolic soil by lead. In Table I, relative sensitivities obtained for different parameters used in the international investigations are summarized. Again, the nitrogen fixing bacteria, soil enzymatic activity (dehydrogenase),





5. Relative sensitivity of N<sub>2</sub>-fixing bacteria, dehydrogenase and respiration activities as indicators of the Pb(NO<sub>3</sub>)<sub>2</sub> contamination in soil

respiration and nitrification/denitrification activity appeared among the most sensitive ones. Since the same parameters are closely linked with ecologically important soil processes of the C and/or N cycles, their alteration may undoubtedly indicate a substantial alteration of soil quality.

The actual task to be further elucidated is that of the limits for the individual stressors. Dahlin et al. (1997) demonstrated this problem recently on soils at low level of metal contamination. An other problem is a remarkable degree of spatial variability of data which can be obtained even for pedogenically homogenous sites (Robertson et al., 1997). Solutions based on statistics have been suggested (Halvorson et al., 1996; Smith et al., 1993) but also alternative methodological developments shall be forced. The latter concerns especially modern molecular-biological and physico-chemical methods in biology, as soon as their practicability will be confirmed.

In the presented conceptual approach to assessing soil quality by biological methods, we attempted to respect the importance of process-related parameters, because the entire biosphere strongly depends on processes of matter biotransformations particularly occurring in soil. Our methodological approach should contribute to the attempted international harmonization in the monitoring of soil quality.

I. Relative sensitivity of the selected microbiological and biochemical parameters for the assessment of soil quality (Evaluation based on long-term soil analyses from 49 differently polluted soil sites)

Parameter	Relative sensitivity
Microbial biomass	*/**
Composition of microbial communities	
Copiotrophic bacteria (Colony forming units)	*/**
Oligotrophic bacteria	**
Actinomycetes	**
Microscopic fungi	**
Proteolytic spore forming bacteria	-/*
Cellulose decomposer	*/**
N <sub>2</sub> -fixing bacteria	****
Pseudomonads	-/*
Biochemical process-linked activities	
Respiration (CO <sub>2</sub> release)	***
Ammonification (NH <sub>4</sub> <sup>+</sup> release)	**
Nitrification/denitrification	**/**
Dehydrogenase activity	***/**
Humification activity	**

Sensitivity (relative to a control soil): - = no detected; \* = low; \*\*\*\* = maximum

## CONCLUSIONS

There is no doubt that soil microorganisms and their biochemical activities have implications for our understanding of ecosystemic processes and management of soil resources. This makes the evaluation of soil quality by feasible microbiological and biochemical parameters desirable (Evdokimova, 1990; Trazar-Cepeda et al., 1998; Tschërko et al., 1996). Nevertheless, there is a lack in an objective methodological approach which could be generally adopted in a long-term monitoring of soil quality. Here we presented and discussed such an ecological approach. It is based mainly on the indication of biological processes of the C and N cycles, but includes also basic aspects of the functional diversity of soil microorganisms. If implemented using internationally adopted and standardized methods, this approach could agree with the requirements of ecologists, different soil users, and environmental policy makers on the monitoring of soil quality.



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#### Návrh a aplikace ekologicky podložených indikátorů kvality půdy.

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Kvalitou půdy rozumíme integrální hodnotu zahrnující půdní strukturu a funkce ve vztahu k účelu, jemuž půda slouží, a k vnějším podmínkám. Přestože na ohrožení

kvality půdy jako nezbytného předpokladu zdravého životního prostředí bylo poukázáno již dříve (Filip, 1973; Kovda, 1975), názor na samovolnou regeneraci půd poškozených antropickými aktivitami dlouho přetrvával. V Evropě bylo možné zaznamenat zásadní přesun v této oblasti teprve koncem osmdesátých a začátkem devadesátých let. Přístup k posuzování a hodnocení kvality půdy se však v řadě zemí do značné míry orientuje kromě nezbytných chemických a fyzikálně-chemických analýz na využívání rozmanitých organismů-indikátorů, jak je to obvyklé v systému ochrany kvality vod. Tento přístup sice umožňuje hodnotit toxický potenciál jednotlivých škodlivin, případně i z hlediska jejich možného vstupu do potravního řetězce, avšak silně zjednodušuje komplexitu půdní biocenózy a zcela opomíjí ekologické funkce půdy.

V předložené práci je poukázáno na vliv, který mají půdní organismy (zejména mikroorganismy) na globální prostředí, obzvláště ve smyslu mineralizace a transformace organických látek v koloběhu uhlíku a dusíku, jakožto procesů nezbytných pro zachování terestrické biocenózy. Je rovněž diskutován možný vliv biodiverzity půdních organismů na ekologické funkce půdy a také vliv chemických polutantů na půdní mikroorganismy a jejich aktivitu.

Z diskuse vyplývá závěr, že pro ekologicky založenou analýzu a hodnocení kvality půdy je nezbytné použít i mikrobiologicko-biochemické parametry. Z našeho hlediska jsou to zejména celková mikrobiální biomasa, ekologicky významné skupiny mikroorganismů a základní biochemicky analyzovatelné procesy transformace organických látek v půdě. Praktické ověření využitelnosti těchto parametrů bylo provedeno v rámci projektu, na němž spolupracovaly skupiny půdních biologů z Německa, České republiky, Maďarska, Ruska a Slovenska. Po zhodnocení více než dvaceti parametrů, které byly aplikovány podle stejné metodiky na půdní vzorky z 49 různě zatížených a využívaných stanovišť, se ukázalo, že zejména mikroorganismy fixující vzdušný dusík, mikrobiální biomasa, aktivita enzymu dehydrogenázy, nitrifikační a denitrifikační aktivita půdy, uvolňování oxidu uhličitého a rovněž schopnost mikroflóry transformovat uhlík susbtrátu přidaného do huminových látek, mohou představovat ekologicky významné parametry vhodné k posouzení kvality půdy. Pro další vývoj hodnotícího systému bude třeba zohlednit variabilitu půd a sezonní variabilitu výsledků analýz a ve stejném smyslu také stanovit limitní hodnoty jednotlivých parametrů.

kvalita půdy; půdní ekologie; mikrobiologické a biochemické indikátory

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