MICROBIOLOGICAL INDICATORS AS IMPORTANT TOOL FOR SOIL QUALITY ASSESSMENT

Daniela RĂDUCU[1], Anca-Rovena LĂCĂTUȘU[1], Irina CALCIU[1], Alina GHERGHINA[1]*, Alexandrina MANEA[1], Olga VIZITIU[1], Petre VOICU[1]

e-mail: alinagherghina@yahoo.com

Abstract

The most significant and appropriate soil indicators are microbiological indicators because they provide a comprehensive picture of the viability of the entire soil ecosystem as well as the quality of the crop. In the Romanian Plain, two Chernozems (P1-irrigated and P2-non-irrigated) were used for the study. The researches presented in this paper belong to a complex study, part of the results (based on the hydrostability indicators) being also published. Thus, the initial premises of the researches were that the results obtained perfectly matched: the microbiological activity should be higher in an ecosystem with a high structural stability and, consequently, with a porous system stable and interconnected. The obtained results emphasized that, according to the microbiological indicators (the number of bacteria; the number of fungi; and the soil respiration), the higher activity of microbiota were in the less stable structural profile, but with higher humidity due to irrigation. In P2, bacteria and fungi, as well as the soil respiration reached higher values. In what concerning the taxonomic identification, in P2, a richness of species, both bacteria and fungi, had been identified, comparing to P1. The microbiological diversity and their distribution in the two soil profiles had been different as a result of the anthropic influence through irrigation. The microbiological indicators use in the paper to emphasize soil quality, proved to be important tools for soil quality assessment and marching with the others important physical indicators, showing also a great accuracy.

Key words: soil quality indicators; microbiology; micromorphology

The most used definition for soil quality (according to the Natural Resources Conservation Service) is the ability of a certain soil to function within a natural or human-used ecosystem, to support the productivity of plants and animals, to preserve or increase the quality of water and air and to ensure the health of living things and the habitat.

Obviously, any change in the ability of the soil to function (respectively in the quality of the soil) will be reflected in the properties of the soil, with corresponding consequences in fertility, economic efficiency, the state of the environment and its biotope.

What is worth pointing out is that soil quality cannot be measured directly, due to its complex characteristics.

Therefore, soil quality can be assessed indirectly, through a series of qualitative and quantitative indicators that describe and quantify both the composition and the structure of the soil, as well as the physical, chemical and biological properties that emphasized the complex processes which shaped the soil in a continuous dynamic and the quality of its life.

The soil quality indicators (SQI), used frequently into the scientific papers are: physical SQI, chemical SQI and biological SQI.

There are several biological soil properties that soil quality can be assessed on the basis of biological properties considered as such or together with physical and chemical properties (Zerihun et al., 2021). Unfortunately, these quality indicators are not universal, but must be considered in relation to the concrete situation in which the assessment is made (Martinez-Salgado M.M., 2010).

When developing sustainable development strategies, it is recommended to consider the soil quality (SQ) indicators that hold degradation factors in various land use and soil management systems (LUSMS) (Bünemanna et al., 2018; Tesfahunegn et al., 2016).

The purpose of this study is to highlight the microbiological indicators (bacteria and fungi number as well as soil respiration) that can be used to evaluate the quality of the soil under irrigated and non-irrigated conditions.

MATERIAL AND METHOD

[1] National Research and Development Institute for Soil Science, Agrochemistry and Environment - ICPA Bucharest, Romania.

The researched had been performed in two sites (P1 – non-irrigated; and P2 – irrigated) located in the Eastern part of the Romanian Plain. The soils are Typic Chernozems (according to SRTS-2012; and Calcic Chernozems according to WRB-SR-2014) formed in loess like deposits.

The climate in the area is temperate continental with an average annual temperature of 10.8 degrees, rainfall of 480 mm. evapotranspiration of 470 mm. The area is characterized by prolonged periods of drought during the summer and early autumn.

The researched area belongs to the steppe bioclimatic zone, where the vegetation (dominated by the associations of *Festuca valesiaca*) had been replaced by the crops with mezo-xerophyle weeds.

Soil samples were taken from each pedogenetic horizon for the microbiological and micromorphological analysis, according to the Research Institute for Pedology and Agrochemistry from Bucharest – Romania Methodology (1987).

Micromorphological investigations were carried out on samples in natural setting, air-dried and impregnated with epoxy resins, on thin sections (25 - 30 μm), studied with the optical microscope in PPL (polarized light) and XPL (cross-polarized light), using the Bullock's terminology (Bullock et al., 1985).

The number of bacteria, fungi and the soil respiration were analized as microbiological indicators.

The number of heterotrophic bacteria (x 10^6 viable cells x g^{-1} dry soil) was determined using the method of dilutions - soil suspensions by dispersion on Topping nutrient medium. The total number of fungi, expressed in x 10^3 cfus x g^{-1} dry soil, was determined by dispersing soil dilutions-suspensions on the Czapek-Dox nutrient medium.

Taxonomic identifications were made according to the manual of determinative bacteriology (Bergey D. H., 1994) and fungi in agricultural soils (Domsch K. H., Gams W., 1972).

Soil respiration (mg CO_2 x g^{-1} dry soil) was determined by the Ştefanic method.

RESULTS AND DISCUSSIONS

The researches presented in this paper belongs to a complex study, part of the results being also published (Răducu D., et al., 2022).

The most significant and appropriate soil indicators are microbiological indicators because they provide a comprehensive picture of the viability of the entire soil ecosystem as well as the quality of the crop. In this respect, the microbiological indicators analyzed had been the number of bacteria and fungi and soil respiration.

The quantitative results of the microbiological analysis showed, in **P1 – non-irrigated** (*figure 1*), a medium (14.68 x 10^6 viable cells x g^{-1} d.s.) total counts of ***bacteria*** in the top Ap horizon, which highly increased to 34.21 x 10^6 viable cells x g^{-1} d.s. in the lower Apt horizon of the tilled layer.

The quantitative findings of the microbiological analysis revealed that the top Ap horizon of the **non-irrigated P1** layer had a medium total bacterial count (14.68 x 106 viable cells x g^{-1} d.s.), which significantly increased to 34.21 x 106 viable cells x g^{-1} d.s. in the lower Apt horizon of the tilled layer (*figure 1*).

The total amount of fungi in the tilled layer was medium, with 69.83 x 103 CFU g^{-1} d.s. in the Ap horizon and 63.38 x 103 CFU g^{-1} d.s. in the Apt horizon, respectively.

These values, which ranged from 21.22 x 103 CFU g^{-1} d.s. to 29.91 x 103 CFU g^{-1} d.s., sharply reduced in the underlying horizons.

As a general indicator of soil microbiological activity, the data for **soil respiration** showed a medium level of activity, the data values ranging from 51.315 mg CO_2 x g^{-1} d.s. to 54.436 mg CO_2 x g^{-1} d.s.

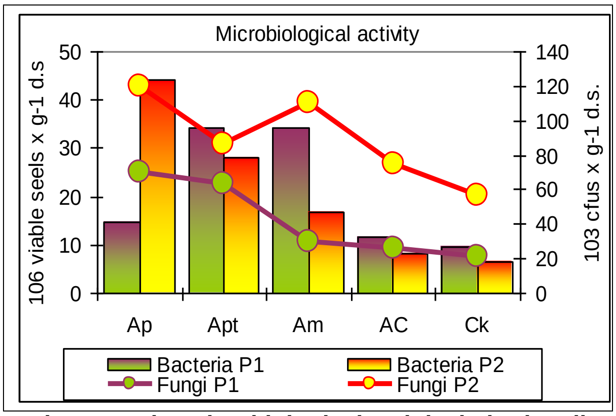

Figure 1. The microbiological activity in both soil (P1 – non-irrigated and P2 – irrigated).

Regarding the **investigations of P2 – irrigated**, the values of the total count of ***bacteria*** were high (44.29 x 10^6 viable cells x g^{-1} d.s.) in the top Ap horizon, and further drastically decreased to low values (6.58 x 10^6 viable cells x g^{-1} d.s.) in the deeper horizons.

The total number of ***fungi*** decreased from the surface, from the high values (120.03 x 10^3 CFU g-1 d.s.), throughout the deeper horizons, where their value was medium (56.68 x 10^3 CFU g-1 d.s.).

The ***soil respiration*** attended high value (67.007 mg CO_2 x g^{-1} d.s.) in the top Ap horizon and slowly decreased (to 45.802 mg CO_2 x g^{-1} d.s.) towards the bottom profile.

The obtained data (*figure 1*) showed that in the irrigated profile (P2), both bacteria and fungi reached higher values (comparing to P1 – non-irrigated). The more compacted Apt horizon is an exception, due to the lower total number of bacteria (comparing to P1).

Into the more compacted Apt horizon, the fungi also decreased, but the total count number still remains higher than in P1.

In both profiles, the values of the total count of both microorganisms showed a general trend of decreasing from the surface toward the deeper horizons.

The fungi in P2 make exception: in the top Ap horizon the higher value (120.03 x 10^3 cfus x g^{-1} d.s.) is attended, but drastically decreased in the more compacted Apt horizon (to 86.61 x 10^3 CFU g-1 d.s.), and increased again into the Am horizon (110.42 x 10^3 CFU g-1 d.s.), where the ecological conditions are best. In the deeper horizons, the fungi decreased again.

The ***taxonomic identification of bacteria*** in relation to the qualitative results (according to the manual of determinative bacteriology of Bergey, 1994) from P1 (*figure 2*) had been showed the dominance of the bacilli group, as well as of the *Pseudomonas*. Moreover, the main genus of *Bacillus* identified in P1 was: *B. megaterium*, *B. cereus*, *B. circulans*, and *Bacillus cereus* var. *mycoides*. With respect to the tilled layer (corresponding to Ap and Apt horizons) the same genus had been identified (such as: *B. megaterium*, *B. cereus*, and *B. circulans*).

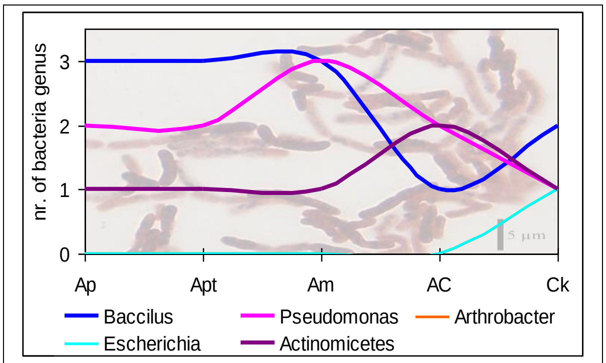

Figure 2. The bacteria taxonomic identification in P1 – non-irrigated.

Concerning the *Pseudomonas* presence in the tilled layer, the identified genus has been: *P. fluorescens* and *P. spp*.

Furthermore, in Am horizon *Pseudomonas pseudogleyi* also appear, enriching the number of genus.

The Actinomycetes, were also identified both in the tilled layer and in the deeper horizons, with the series: *Albus Ruber*, *Fuscus*, and *Flavus*.

In the bottom profile (Ck horizon respectively), the bacilli group, *Pseudomonas*, and Actinomycetes, together with *Arthrobacter citreus* and *Escherichia freundii* were also detected.

In P2, the ***taxonomic identification of bacteria*** highlighted both the presence of more species and their different distribution into the soil profile (*figure 3*), compared to P1.

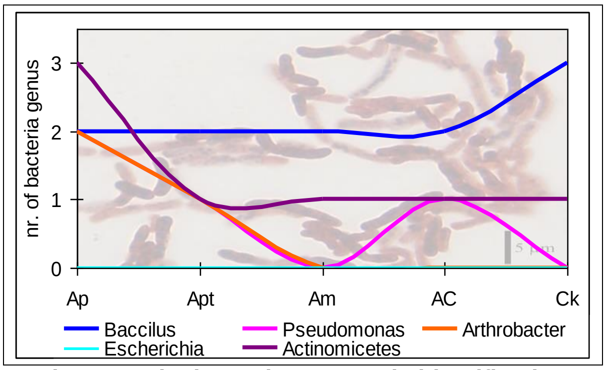

Figure 3. The bacteria taxonomic identification in P2 – irrigated.

Thus, into the Ap horizon of the tilled layer, the identified bacteria had been: *Micrococcus* spp., *Pseudomonas fluorescens*, *Arthrobacter globiformis*, *Xanthomonas pseudogleyi*, *Bacillus megaterium*, *Bacillus circulans*, *Pseudomonas lemonnieri*, *Arthrobacter citreus*, and Actinomycetes (Series *Albus*, *Luteus*, and *Fuscus*).

In this respect, the more abundant bacteria in the top horizon of the P2 – irrigated, showed better living conditions. Even better than into the Am horizon, where the bacteria identified had been: *Bacillus mesentericus*, and *Bacillus circulans*, together with the Actinomycetes S. *Albus*.

In Apt horizon, even if more compacted and implicitly less porous, the bacteria identified were, in decreased order: *Bacillus circulans*, *Pseudomonas* spp., *Bacillus megaterium*, *Arthrobacter globiformis*, and Actinomycetes S. *Albus*.

In the deeper horizon of P2 (Ck horizon), the number of bacteria genus is also high: *Bacillus megaterium*, *Bacillus cereus*, *Bacillus circulans*, *Pseudomonas* spp., *Micrococcus* spp., and Actinomycetes with *Fuscus*, and *Albus* Series).

Into the studied profiles, either non-irrigated, or irrigated, *Pseudomonas* is among the most dominant bacteria.

Rhizobacterium *Pseudomonas fluorescens* has a variety of traits that allow it to function as a biocontrol agent and to encourage plant growth. *Pseudomonas fluorescens* competes aggressively with other microorganisms, adapts to environmental stresses, and also produces a wide range of bioactive metabolites (antibiotics, siderophores, volatiles, and growth-promoting substances) in the plant rhizosphere. In addition, they are in charge of the natural suppressiveness of some soil-borne pathogens (David B.V., et al., 2018).

Although *Bacillus* is, by far, the dominant bacteria in P1 non-irrigated, in P2 irrigated it was no identified, the humidity conditions being higher.

The effects of members of the *Bacillus* genus on plant growth and development have long been recognized. They increase agricultural output by strengthening plant stress tolerance, disease defense, and mineral and water uptake (Zerihun T., et al., 2021). Another crucial factor is connected to *Bacillus* species' ability to build biofilms on plant surfaces, which helped plants tolerate drought better (Zerihun et al., 2021).

Alia S., et al. (2020) pointed out that both *Pseudomonas* and *Bacillus* strains demonstrated the potential to create a biofilm on an artificial surface, allowing them to colonize and benefit the host plant to the fullest extent possible.

By increasing the amount of lateral roots and root hairs, bacterial phytohormone secretion can alter root architecture, which in turn boosts nutrient and water uptake and fosters development (Persello-Cartieaux F., et al., 2003). Plants utilise microbial compounds such exopolysaccharides (EPS), phytohormones, and 1-aminocyclopropane-1-carboxylate (ACC) for drought tolerance. Plants partially rely on soil microorganisms to reduce stress produced by drought (Zerihun T., et al., 2021).

Arthrobacter had been identified into the deeper horizon of P1 non-irrigated, as well as into the surface horizons (both Ap and Apt) of the P2 – irrigated.

One of the most often isolated, native, aerobic bacterial genera found in soils is *Arthrobacter* sp. Members of the genus have a variety of metabolic and ecological traits and can endure severe environments for long periods of time (Mongodin E.F., et al., 2006).

Regarding **the *fungi* taxonomic *identification*** (according to Domsch et Gams, 1972) in both studied profiles (P1 and P2 - *figure 4* and *5*), the number and the distribution of the species and genus, had been different from one profile to another (as in case of bacteria).

In P1 the identified genus of fungi into the tilled layer were (*figure 4*): *Trichoderma harzianum, Fusarium oxysporum, Penicillium verrucosum, Aspergillus ochraceus,* and *Acremonium strictum (*in the top Ap horizon*)*; and *Trichoderma harzianum, Trichoderma viride, Fusarium pallidoroseum,* and *Aspergillus terreus (*into the Apt horizon*)*.

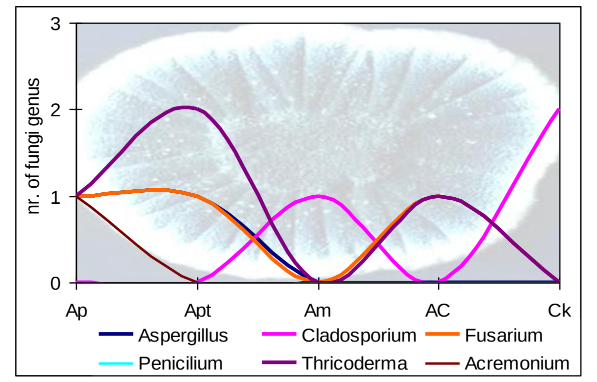

Figure 4. The taxonomic identification of the fungi from P1 – non-irrigated.

In the deeper horizon of P1 – non-irrigated, the number of the fungi species drastically decreased.

Thus, into the mollic (Am) horizon, with the best ecological conditions, only *Cladosporium sphaerospermum* had been identified, while into the AC horizon *Trichoderma harzianum* and *Fusarium solani* were detected. In the deeper Ck horizon, only *Cladosporium herbarum,* and *Cladosporium sphaerospermum* had been observed.

In **P2 – irrigated, the *fungi taxonomic identification*** showed a richness of species, in all the pedogenetic horizons (*figure 5*). Thus, in the top horizon of the soil and in the tilled layer, the identified species of fungi were: *Trichoderma viride, Trichoderma harzianum, Penicillium verrucosum, Chaetomium globosum, Penicillium commune, Penicillium frequentans, Aspergillus ustus, Mucor hiemalis,* and *Eurotium herbariorum*.

Into the Apt horizon, the species number slightly decreases: *Fusarium culmorum, Cladosporium herbarum, Aspergillus terreus, Aspergillus flavus, Penicillium* sp., and *Penicillium frequentans*.

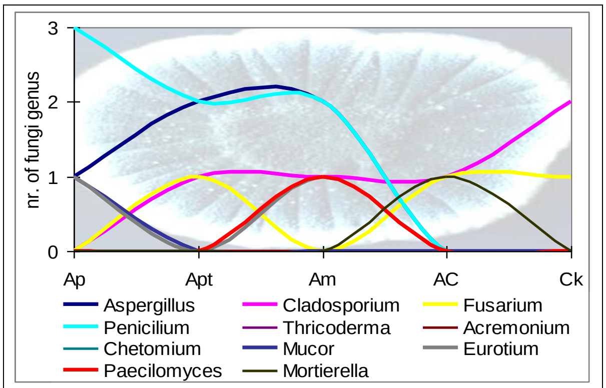

Figure 5. The taxonomic identification of the fungi from P2 – irrigated.

Mollic horizon, rich in organic matter, had been favored the development of: *Aspergillus tamarii, Penicillium* sp., *Cladosporium herbarum, Paecilomyces variotii, Eurotium herbariorum, Aspergillus terreus,* and *Penicillium* sp.

Into the deeper horizons, the fungi were still abundant: into the AC horizon *Fusarium oxysporum, Cladosporium cladosporioides, and Mortierella isabellina* also developed.

In the bottom profile, the number of fungi genus maintained to a relatively high level, thus, *Cladosporium sphaerospermum, Fusarium verticillioides, Cladosporium macrocarpum*, and *Mortierella isabellina, Paecilomyces elegans, and Cladosporium herbarum* were identified.

According to studies, "biofilm" is crucial to both the development of plant diseases and beneficial interactions (Lugtenberg B., Kamilova F., 2009).

By ensuring a strong root system, maintaining rhizosphere moisture, and adjusting soil pH, it can give organisms a way to establish and maintain themselves in a favorable environment, benefit from nutrients secreted by the plants, and influence them directly or indirectly, resulting in improved nutrient cycling (Davey M.E., O'Toole G.A., 2000).

Răducu D., et al. (2022), studying the structural vulnerability (using the structural hydrostability indicators' objective) of the Chernozems approached in this paper, showed that in P2-irrigated, due to their friability, the aggregates disintegrated when subjected to physico-mechanical operations. Consequently, an instable pore system formed.

The initial premises of this paper researches were that the microbiological indicators results should perfectly matched with the hydrostability indicators values (Răducu D., et al., 2022): the microbiological activity should be higher in an ecosystem with a high structural stability and, consequently, with a porous system stable and interconnected. But the obtained results pointed out that, according to microbiological indicators, the higher activity of microbiota were in the less stable structural profile, but with the higher humidity (in the P2-irrigated respectively). It was obvious that for the microorganisms, the soil humidity represented the crucial factor for their development (for their number as well as for their species and genus richness).

The microbiological diversity and their distribution in the two soil profiles (non-irrigated and irrigated) had been different as a result of the anthropic influence through irrigation.

The microbiological indicators use in the paper to emphasize soil quality, proved to be important tools for soil quality assessment and marching with the others important physical indicators, showing also a great accuracy.

CONCLUSIONS

The obtained results based on the study of microbiological indicators in different conditions (non-irrigated and irrigated), were different of the initial premises of the researches: best higher activity in best structured soil (with high and stable porosity).

The obtained data showed that in P2 – irrigated, higher values were attained for the soil respiration, bacteria, and fungi, comparing to P1 – non-irrigated, except the Apt horizon where the total number of bacteria is lower (than in P1), and the fungi also decrease, but the total count number still remains higher than in P1.

On the other hand, the taxonomic identification highlighted in P2 – irrigated a richness of species, both for bacteria and fungi, in all the pedogenetic horizons, comparing to P1 – non-irrigated.

The number of bacteria, the number of fungi and the soil respiration proved to be important and accurate indicators for soil quality assessment in both ecological different conditions of non-irrigated and irrigated.

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