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MICROBIOLOGICAL AND ENZYMATIC ACTIVITY OF SOILS IN THE VICINITY OF A WIND TURBINE

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ABSTRACT

There have been microbiological and enzymatic analyses of soils located at different distances and in different directions from a wind generator (0 m, 20 m, 50 m, 1000 m) performed, in order to establish the impact of wind on the indicators researched. The biogenousity of soils is different for the individual samples, the total microflora is highest when the soil most heavily influenced by the wind – at a distance of 20 m from the wind turbine into the wind. Overall, the highest percentage share in the composition of the soil occupy the ammonification bacteria (non-spore forming bacteria and germs), followed by actinomycetes and micromicetes. The mineralization factor is lowest in the sample with the highest amount of micro-organisms and the highest in the samples against the wind direction and control. The cellulose activity is not limited by the wind erosion and its consequences – it is highest in soils in the direction of the wind and lower in the soil against the wind direction and the control point. Unlike it the catalase activity shows almost the same values in all soils tested. The microbiological and enzymatic indicators alone or in combination can serve as sensitive biological and biochemical indicators in the study of soils in the vicinity of wind turbines or influenced by wind erosion.

KEYWORDS - Actinomycetes, Ammonification bacteria, Bacteria, absorbing mineral nitrogen, Catalase, Celullase, Micromycetes

1. INTRODUCTION

The increase in the global energy consumption leads to the use of renewable energy sources. According to Armstrong et al. (2014) [1] the wind turbines and photovoltaic panels can alter the microclimate in large scale, so soil processes and vegetation cover to be affected. The soil is recognized as the largest source of ground organic carbon, where its contents is more than that in the vegetation and the atmosphere [2]. The effects of wind and solar parks on local climate can change the cycle of carbon directly through changes in the temperature of the air and soil, the rainfall and evaporation (and hence also soil moisture) and the balance of direct and diffuse radiation [3,4,5,6,7]. The consequences can not only be direct but also indirect – the climate changes induced lead to changes in the composition of plants, soil and microbiotic activity of the soil micro-organisms.

As a result of the wind erosion the soil micro-organisms are affected in three areas: (1) loss of certain microbial taxa, depending on the size of the soil particles and wind conditions, (2) through the destabilization of the soil aggregates and reducing of the available surfaces, and (3) through the reduction of organic matter and substrates [8]. The transport of particles by the wind is often over great distances [9,10], which are diverse in composition - mineral and organic materials, biochemicals, xenobiotics (such as pesticides) and micro-organisms. Of these components, the microbial composition in the dust is the least explored, which is partly due to the greater abundance and diversity of microorganisms in the soil.

The microorganisms play a key role in protecting the soils from erosion through their influence in the formation of the organic matter and the construction of a stable soil aggregates. Thus, practices that increase soil microbial biomass and carbon content, will contribute to the improvement of the soil structure and reduce the soil erosion. Growth of the micromicetes, for example, leads to the physical connection of the aggregates and the production of glomalin-agents, which act as the glue soil particles [11]. An increase in the soil organic matter, which subsequently leads to the improved stability of the soil aggregates and increase in the water retaining ability of the soil is the result of the activity of fungi and bacteria. Soil fungi, and to some extent actinomycetes, degrade the complex compounds to simpler forms that are consistently used by the bacteria. Bacterial species included in symbiotic associations of nitrogen fixation (i.e. rhizobia) and fungal species (for example, from the type *Glomeromycota*) are able to maintain mikorrhizal associations, that increase the moisture and assimilable phosphorus in the soil. In addition to bacteria and fungi, it was found arhei to play a large role in the nytrification and thus they are also an important microbial group for the nitrogen cycle and the performance of the agri-ecosystems [12].

The changes in the microbial and biochemical properties may represent early and sensitive indicators for management of induced modifications regarding the quality of the soil, as they manifest themselves for shorter time periods compared to the decomposition of organic matter [13,14,15]. The research aimed at establishing the influence of wind erosion on the enzymatic activity of soils are very little. In the future in relation to the research of the influence of the wind erosion on microbiological and enzymatic activity of soils additional approaches are expected that could provide information not only to identify the microbes but also to determine their activity in soils subjected to dust pollution from wind [16,17]. Such a focus will address the persistent gaps in the knowledge about the role of different microbes in the environment, especially in maintaining the functions of the soil [18,19]. Methods that use RNA to determine the diversity of the micro-organisms and their activity in soils subjected to wind erosion are promising [8].

The purpose of this research is to trace the influence of the wind on the microbiological and enzymatic activity of soils located at a different distance from the wind turbine.

2. MATERIAL AND METHODS

The soils are sample collected from the land of Gorichane village, Shabla Municipality. Dobrich in August 2015, at a different distance and direction of the "Micon" model wind turbine, with the following characteristics:

- Power 400 kW;
- Tower height – 36 m;
- Wind turbine rotor diameter – 27 m;
- Wind speed turn on – 3-4 m/s;
- Wind speed turn off – 25 m/s;
- Rotor blades number – 3.

The following samples were taken:

1. Control point – 1000 m from the wind turbine;
2. Next to the wind turbine – 0 m in the direction of the wind;
3. 20 m from the wind turbine, in the direction of the wind;
4. 50 m from the wind turbine, in the direction of the wind;
5. Next to the wind turbine – 0 m, against the direction of the wind;
6. Next to the wind turbine – 50 m, against the direction of the wind.

The samples for microbiological analysis were taken with a sterile tool, in sterile paper bags. They were transported and tested not later than 48 hours, until the moment of the test culture they were stored in a refrigerator at 4° to 10°C. The microbiological studies include defining ammonification bacteria (non-spore forming bacteria and germs), actinomycetes, micromycetes, bacteria absorbing mineral nitrogen. They are determined by the method of dilution and inoculation on solid media (mesopeptic agar – to determine the non-spore forming bacteria and germs; starch-ammonium – agar for determination of actinomycetes and bacteria, absorbing mineral nitrogen; environment of Capek-Dox – for determining micromycetes), their cultivation in a thermostat and subsequent reporting of colony forming units, converted to 1 g absolutely dry soil.

The statistical processing of the data of the microbiological indicators include the calculation of the average value from three repetitions and variant coefficient.

The cellulose activity of the samples was tested through laboratory experience [20]. In a Petri dish, soil with around 7 mm in thickness is poured while maintaining 60% PPV. Over the soil on 3 sterile filter paper strips with dimensions of 10/50 mm are placed. Cultivation in a thermostat at 25°C. Through 15 days accounted is the rate area deteriorated with the help of a reference network sample. The catalase activity of the soil is determined through the mangano-metric method [20].

The statistical processing of the data from the catalase activity involves the calculation of the average value from three repetitions and standard deviation.

3. RESULTS AND DISCUSSION

The biogenousity (total microflora) of the soil samples is calculated through a summary from the total amount of non-spore forming bacteria, germs, actinomycetes and micromycetes. It is different for the different objects. The results of the microbiological analysis are presented in table 1.

It's visible from the table that the total microflora is highest at 20 m from the wind turbine and the lowest at 50 m from the wind turbine, in the direction of the wind. The wind erosion leads to the removal of part of the surface soil and its accumulation elsewhere. In this way, micro-organisms are also transferred and are adsorbed on the surface of the soil particles. In the air they can't move actively and multiply, but wear on dust or in microscopic droplets. Probably the wind speed remains high up to 50 m from the wind turbine, which lays down the most powerful impact on the wind erosion for the removal of part of the superficial soil layer and the quantity of microorganisms is then reduced. This trend is confirmed by the fact that, at the object against the direction of the wind at the same distance (50 m), as well as in the control point the total microflora is 4 times more. The micro-organisms need, if not from a larger quantity of water, from moisture at least. The wind also contributes to the drying of the soil and reducing of their temperature. But the drying and the frost predicted not always kills the micro-organisms. Many bacteria tolerate drying out as they induce very slow metabolism. When they dampen again, they recover their usual vibrant activity. In the same way the bacteria tolerate cold and food shortages. Sometimes the stress conditions lead to the development of the micro-organism activation as a response to the adverse environment achieved. Another time these conditions inhibit the strong development of the micro-organisms.

Table 1: Qualitative And Quantitative Composition Of The Microorganisms (Cfu X 10^3/G Abs. Dry Soil) ± C.V.; (%)

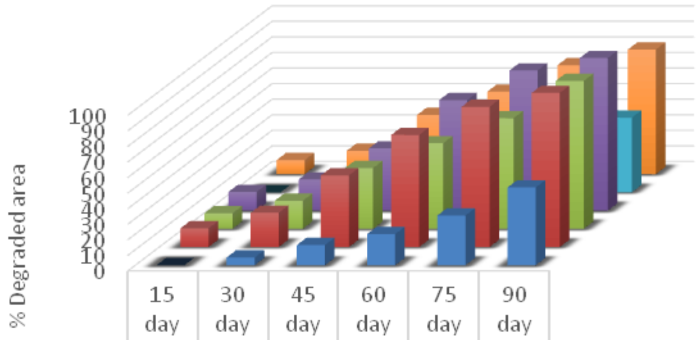
Object	Depth (cm)	General number microorganisms	Non-spore forming bacteria	Germs	Micro-mycetes	Actino-mycetes	Bacteria absorbing mineral nitrogen	Mineralization coefficient
Control point – 1000 m from the wind turbine	0-10	7940	6400±0,156 (80,6)	760±2,279 (9,6)	280±1,237 (3,5)	500±1,600 (6,3)	8960±0,511	1,25
Next to the wind turbine – 0 m in the direction of the wind	0-10	6840	4160±0,192 (60,8)	1020±1,698 (14,9)	660±1,515 (9,6)	1000±1,000 (14,6)	3360±1,190	0,65
20 m from the wind turbine, in the direction of the wind	0-10	17280	16320±0,055 (94,4)	180±5,556 (1,0)	380±2,632 (2,2)	400±1,000 (2,3)	5760±0,694	0,35
50 m from the wind turbine, in the direction of the wind	0-10	2060	800±1,250 (38,8)	500±2,000 (24,3)	280±3,571 (13,6)	480±2,083 (23,3)	1360±2,941	1,05
Next to the wind turbine – 0 m, against the direction of the wind	0-10	4030	1580±1,675 (39,2)	920±1,087 (22,8)	660±0,758 (16,4)	860±1,163 (21,6)	3840±1,042	1,54
Next to the wind turbine – 50 m, against the direction of the wind	0-10	8800	6240±0,424 (70,9)	640±1,563 (7,3)	400±2,500 (4,5)	1520±0,658 (17,3)	7840±0,460	1,14

The highest percentage quota of the total microflora in all tested soils occupy the non-spore forming bacteria, as their amount is highest in the sample of 20 m from the wind turbine into the wind (94,4%) and lowest in the sample of 50 m from the wind turbine into the wind (38,8%). High is the quantity of the micro-organisms in the control group sample (80,6%), taken at a distance of 1000 m from the wind turbine. Lower than the control point is their content in the sample taken immediately next to the wind turbine – 60,8% – respectively in the sample in the direction of the wind, and 39,2% in the sample against the wind direction. The amount of the non-spore forming bacteria in the soil at 50 m away from the wind generator against the wind direction is close to that in the control point, such as the relative percentage share of the total microflora was 70,9%. This trend shows that in the sample of 20 m away from the wind turbine the high wind speed increases the development of non-spore forming bacteria. Probably, the continuous movement of the air masses results in better aeration of the superficial layer, to export, but also to the introduction of nutrients, to changes in the moisture content in the soil.

The second highest percentage quota in relation to the total quantity of microorganisms are the germs, with the exception of the sample at 20 m from the wind turbine into the direction of the wind and that at 50 m downwind. These two groups of micro-organisms play a key role in the initial stages of destruction of the organic matter in the soils. The quantity of actinomycetes is close to that of germs and above them under the two shown before samples. The most weak represented in the composition of total microflora are the micromycetes which develop better with higher humidity. Polyanskaya and Zvyagintsev (2005) [21] found out that the anthropogenic factors strongly influence the biomass of fungi and, to a lesser extent the biomass of bacteria. Apparently the high wind speed in the sample of 20 m away from the wind turbine into the wind direction, as well as the low wind speed in the sample of 50 m away from the wind generator against the wind direction create different conditions for the development of micro-organisms to which they have adapted. With these two samples is established the highest total amount of microorganisms and a change in the percentage share of the total microflora in comparison with the other samples. According to Krasilnikov (1958) [22], the distribution of the bacterial cells in the soil is diffuse, and in individual ones more or less limited outbreaks. These specific habitats advantage the development of micro-organisms. In the event of changes to the ecosystems, such as the receipt of fresh organic matter, fertilizer application or following of contaminants in the soil, in the processes of their transformation microorganisms are included that need to bring the system into a state of equilibrium.

In terms of the bacteria, absorbing mineral nitrogen, their content is highest at the control point, followed by that in the sample of 50 m away from the wind turbine against the wind direction. The lowest level is the quantity in this group of soil microorganisms of 50 m away from the wind turbine into the wind. The ratio between the bacteria, absorbing mineral nitrogen and the sum of the non-spore forming bacteria and germs determine the mineralization factor. This parameter defines the rate of decomposition of organic matter in the soil and thus shows the activity of the microorganisms. It is noticed that the highest amount of microorganisms does not determine the increase in their activity, i.e not always the high amount of microorganisms leads to high activity, as in the soil of 20 m from the wind turbine into the wind, whereby the mineralization factor is lowest – 0,35. The highest rate of mineralization in the soil of 0 m from the wind generator against the direction of the wind, as well as in the control point. Therefore the wind erosion has no effect in reducing the amount of microorganisms, and it even goes up, but decreases the activity of the microorganisms in terms of total quantity organic substances.

The cellulase activity shows an opposite trend to that of the mineralization factor – its activity is higher in soil in the wind direction (fig. 1).



	15 day	30 day	45 day	60 day	75 day	90 day
	% Degraded area					
Control point – 1000 m from the wind turbine	0	5	13	20	32	50
Next to the wind turbine – 0 m in the direction of the wind	12	22	46	72	90	99
20 m from the wind turbine, in the direction of the wind	10	18	39	55	71	95
50 m from the wind turbine, in the direction of the wind	12	20	40	71	90	98
Next to the wind turbine – 0 m, against the direction of the wind	0	4	10	18	25	48
Next to the wind turbine – 50 m, against the direction of the wind	9	15	38	53	70	80

Fig. 1: Cellulase Activity Of Soil Microorganisms

We can see from the graph that the cellulase activity is higher in the soil next to the wind turbine and at 20 m from it into the wind, which is depending on the increased amount of total microflora in these soils. High is the percentage of the unfenced area is (98% on the 90-day from reporting), however, and in the soil with the lowest amount of microorganisms – the sample of 50 m from the wind turbine into the wind. Therefore, once again the trend is confirmed that not always, and not only the high quantity of microorganisms is subject to their activity. The activity and structure of microbial communities in the soil may be affected by some factors, such as carbon source, available nutrients, presence of water, pH, soil temperature, soil microorganisms interactions between themselves [23,24,25]. These factors are variable and therefore the so-called micro-locality presence in soils are dynamic systems.

Lowest is the cellulase activity in the control sample, which is 1000 m from the wind turbine (50%), as well as in both samples downwind – respectively to the wind turbine (48%) and 50 m from the wind turbine (80%). Therefore, the wind has no negative impact on the activity of the cellulase, and it even intensifies. However, it can lead to drying of the soil. The stress factor has an influence on the development of different bacterial communities. Altimirska et al. (2006) [26] did a research on drought tolerant soybean genotypes and the microbiological activity in the soybean rhizosphere. They found that drought, as an important abiotic factor is of particular importance as regards to the productivity of soybeans, and in terms of the microbiological activity in the soil. The studies on the influence of drying and over dampening of soils as a stress factor for the development of microorganisms are numerous [27,28,29,30,31,32,33,34,35,36].

Unlike the cellulase activity, the catalase shows almost the same values in all soils tested (table 2).

Table 2: Catalase Activity Of The Soil Microorganisms (Ml 0_2/30 Min.)

Object	Depth (cm)	ml 0_2/30 min. Mean ± SD
Control point – 1000 m from the wind turbine	0-10	1,35±0,050
Next to the wind turbine – 0 m in the direction of the wind	0-10	1,35±0,100
20 m from the wind turbine, in the direction of the wind	0-10	1,40±0,100

Next to the wind turbine – 0 m, against the direction of the wind	0-10	1,45±0,087
Next to the wind turbine – 0 m, against the direction of the wind	0-10	1,40±0,150
Next to the wind turbine – 50 m, against the direction of the wind	0-10	1,40±0,200

The catalase activity varies from 1,35 to 1,45 ml 0_2/30 min in the studied samples, which indicates that it cannot serve alone as a sensitive indicator for soils near the wind turbine. This trend, however, is required to be bound to studies in dynamics, as well as studies of other factors influencing the activity of the enzyme. Grozeva and Nustorova (1995) [37] in the study of the catalase activity and some chemical properties of Mediterranean soils, found that the catalase activity correlates with the contents of iron, hygroscopic humidity and in most cases with the humus in the soil. According to them, the activity of catalase probably depends on the organic fraction of the soil, and its mineral part (non-enzyme catalysts). The catalase activity of the soil depends on the soil type, presence of inhibitors, and methods of investigation. It is determined by mineral and organic soil part dead, according to researches of Kuprevich and Shcherbakova (1966) [38] reaching up to 14% of the total soil activity. Kappen (1972) [39] establishes also a residual catalytic activity which reaches 84% of the starting enzyme activity.

4. CONCLUSIONS

The total quantity of microorganisms is the highest in the soil most heavily influenced by wind (20 m in the direction of the wind). The lowest is the total microflora in the soils in the immediate vicinity of the wind turbine, as well as in the sample of 50 m downwind, where its speed is lower.

The highest percentage share in the composition of the soil's microflora occupy the non-spore forming bacteria and germs (with the exception of the sample at 20 m away from the wind turbine into the wind), and the least presented are the micromycetes.

The mineralization factor is lowest in the sample with the highest amount of microorganisms (20 m from the wind turbine into the wind) and highest in samples against the wind direction and control point.

The celulase shows the opposite trend of the mineralization factor – higher is its activity in the soils in the direction of the wind and the lower in the soil against the wind direction and control point. Therefore, the celulase activity is not limited by wind erosion and its consequences.

Unlike the cellulase activity, the catalase shows almost the same values in all soils tested. The activity of the enzyme is probably determined by other factors – the iron content, moisture, organic and mineral portion of soil as well as by the presence of catalase with plant origin.

The microbiological and enzymatic indicators alone or in combination can serve as specific biological and biochemical markers in the study of soils in the vicinity of the wind turbines and influenced by the wind harmful consequences to the soil – changing its physical, chemical and biological properties.

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