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Effect of oxygen deficiency on soil dehydrogenase activity (pot experiment with barley)

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A b s t r a c t. Changes of dehydrogenase activity in Orthic Luvisol developed from loess (Ap horizon) under different aeration conditions were observed in a greenhouse pot experiment with barley *Hordeum vulgare* L. cv. Aramir vegetation. The aeration status was modi- fied by the use of combinations of three soil bulk densities (1.20, 1.35 and 1.50 Mg m^{-3}) and three levels of soil water status (i - the suction range of 15-80 kPa as the control level; ii - the suction range of 2-5 kPa and iii - water saturation). All the combinations of water conditions and bulk density were replicated in four pots. The plants were grown in soil with a controlled water level, except during 14-days of oxygen stresses which were applied at three plant physiological stages by reducing the water suction to 2-5 kPa or to 0 kPa (water saturation). Oxygen stresses were imposed as follows: stress I - at tillering; stress II – at shooting, and stress III - at the beginning of plant flowering. The aeration parameters (oxygen diffusion rate – ODR, redox potential – Eh, concentration of Fe^{+2}) as well as soil dehydrogenase activity were measured four times during each stress. The dynamic of dehydrogenase activity is shown together with natural changes of temperature. Significant correlations between dehydrogenase activity and aeration indexes (Eg, ODR, Eh, Fe^{+2}) were observed.

K e y w o r d s: dehydrogenase activity, soil aeration parameters, barley, oxygen diffusion rate, redox potential

INTRODUCTION

Dehydrogenase activity is commonly used as an indicator of biological activity in soil [3,14,22]. Dehydrogenase enzymes play a significant role in the biological oxidation of soil organic matter by transferring protons and electrons from substrates to acceptors. These processes, being a part of the respiration pathways of soil micro-organisms, are closely related to soil aeration [2,5,9,10,19]. The status of soil oxygenation depends on air-water conditions and influences plant growth [11,15,20].

The objectives of the present study were the evaluation of the influence of the soil's physical factors such as water status and bulk density on dehydrogenase activity and to determine the relationship between the enzyme activity and soil aeration parameters in a pot experiment with barley vegetation.

MATERIAL AND METHODS

The soil (Orthic Luvisol developed from loess, Ap horizon, Table 1) was sieved without drying through a 0.5 cm

T a b l e 1. Basic characteristics of the Orthic Luvisol

C org (%)	Particle size (mm) distribution (%)			Particle density (Mg m^{-3})	pH H_2O
	1-0.05	0.05-0.002	<0.002		
1.54	25	70	5	2.58	6.5

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sieve. Plastic pots (6 dm^3 volume, 20 cm high) were filled with the soil material and three levels of soil compaction: 1.20, 1.35 and 1.50 Mg m^{-3} were prepared by manual compaction. Before sowing the seeds, the soil was fertilised with 0.1 g N, 0.125 g K and 0.066 g P kg dry $soil^{-1}$ in the form of NH_4NO_3, K_2SO_4 and KCl, and $CaHPO_4$ $2H_2O$, respectively. Barley seeds (*Hordeum vulgare* L. cv. Aramir) were sown at a depth of 2 cm. The seedlings were pricked out after emergence to 25 plants per pot.

The experiment was conducted in a greenhouse. Soil water suction was maintained within the range of 15-80 kPa in all the pots, except during the period of oxygen stresses, when air-water conditions were modified. Soil water suction during stress was reduced to 2-5 kPa or to 0 kPa (5-10 mm water layer on the soil surface). This change of soil water status was made for each bulk density treatment. However, sufficient pots remained at a suction of 15-80 kPa as controls. Four pots for each combination of water level and density were prepared as replicates. Oxygen stresses (oxygen deficiency periods) were imposed at three plant physiological stages:
stress I - at tillering,
stress II - at shooting,
stress III - at beginning of flowering.

A complete set of 36 pots was used for each stress. The periods of modified soil aeration were maintained for 14 days and soil analyses were made four times.

Before soil sampling, redox potential (Eh) and oxygen diffusion rate (ODR) by the use of Pt electrodes as well as pH in situ by the use of combined glass electrode were measured [6,11,16].

Soil was taken from three places in each pot (0-5 cm) and mixed carefully. Measurements of Fe^{+2} content were made in sulphuric acid extracts with the use of α, α'- dipyridyl in acetate buffer, pH 4.5 [1]. Dehydrogenase activity was determined with TTC (triphenyl tetrazolium chloride) according to a modified method of Casida *et al.* [4] and expressed as nmol TPF (triphenyl formazan) g^{-1} oven dry soil min^{-1}. The content of Fe^{+2} and the dehydrogenase activity were calculated on the basis of the oven-dry (105°C) soil mass.

RESULTS AND DISCUSSION

Figure 1 presents the changes of dehydrogenase activity during three vegetation stages under oxygen stresses. The values of enzyme activity at particular water status were the average values of three degrees of compaction. Dehydrogenase activity remained unchanged in control soil (15-80 kPa) and slight changes at water regime of 2-5 kPa were observed. However, water saturation (0 kPa) resulted in elevation of dehydrogenase activity, especially between the 3rd and 14th days of stress II, performed at the stage of plant shooting. This strong increase in enzyme activity was contemporaneous with the highest temperature (up to 28°C) for the vegetation season.

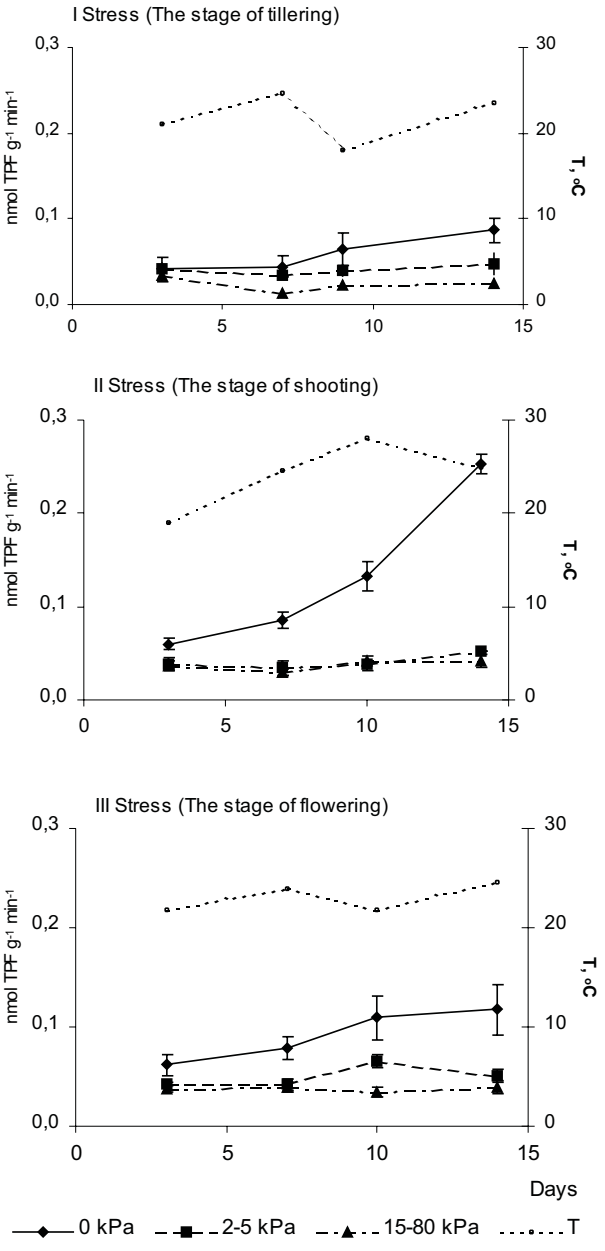

Fig. 1. Dynamic of dehydrogenase activity of Orthic Luvisol developed from loess during oxygen stresses at three physiological stages of barley vegetation (average values of three soil densities). Errors bars are standard deviations of the means.

Figure 2 shows dehydrogenase activity for particular levels of soil water conditions and bulk density, expressed as average values for the entire period of the experiment. Analysis of variance showed significant influence of both the parameters ($P<0.001$). The density factor caused an increase in activity averaging 25% but water status – averaged

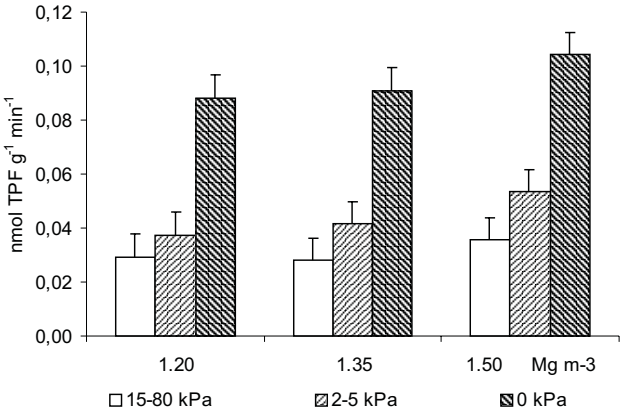

Fig. 2. Dehydrogenase activity of Orthic Luvisol at particular combinations of water status and degree of compaction (average values of the entire experiment and 95% confidence half intervals of Tukey).

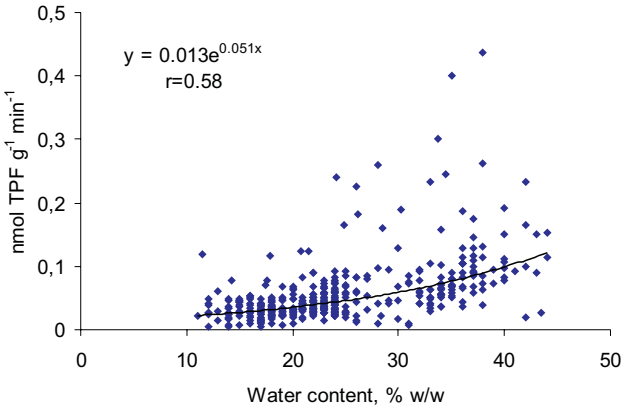

Fig. 3. Dehydrogenase activity versus soil water content (results of entire experiment included).

3-fold. The soil with low bulk density and control water conditions (1.2 Mg m^{-3}, 15-80 kPa) showed the lowest activity (in average 0.029 nmol TPF g^{-1} min^{-1}), and that with both high density and water saturation (1.5 Mg m^{-3}, 0 kPa) - the highest activity (in average 0.104 nmol TPF g^{-1} min^{-1}).

The increase of the dehydrogenase activity was connected with the change of the soil aeration status. Modification of soil conditions from well-aerated to water-saturated soil implicates several physical-chemical and biological processes [7,8,11-13]. The shift of activity from aerobic to anaerobic micro-organisms, following the depletion of O_2 after soil flooding, is accompanied by a decrease of redox potential, alteration of pH and increases of the concentrations of reduced mineral forms (NH_4^+, Fe^{+2}, Mn^{+2}, S^{-2}). A biochemical consequence of these transformations is an increase in the activity of soil dehydrogenases [2,9,17,18].

Figures 3-8 show the relationships between soil dehydrogenase activity and some soil indexes connected with the air-water regime. All the results obtained in the pot experiment with barley vegetation were included. Soil water content (Fig. 3) was in the range of 10–45% (w/w). The enzyme activity increased with increasing soil water content.

Figures 4-7 present the relationship between the activity of soil dehydrogenases and soil aeration. The O_2 status was expressed by the indexes: Eg, ODR, Eh and Fe^{+2} content. The most appropriate mathematical models were chosen for the particular relations. All the relationships were statistically significant.

Air-filled porosity is the simplest and probably the oldest indicator of soil aeration. Eg values (averages for the entire vegetation season) were equal to 0.24, 0.15 and 0.08 m^3m^{-3} for particular compactions (1.20, 1.35 and 1.50 Mg m^{-3}, respectively). The highest Eg value (0.39 m^3m^{-3}) was

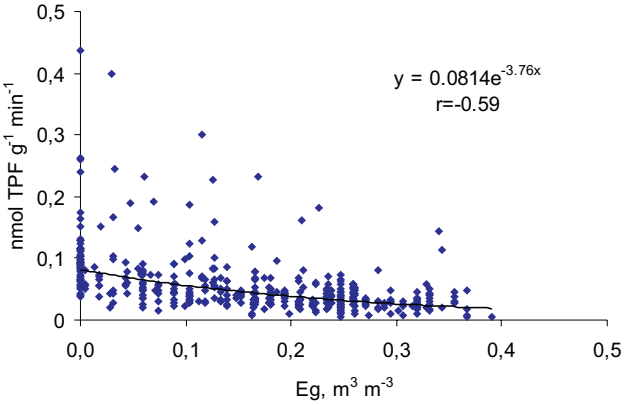

Fig. 4. Relation between soil dehydrogenase activity and air-filled porosity (results of entire experiment included).

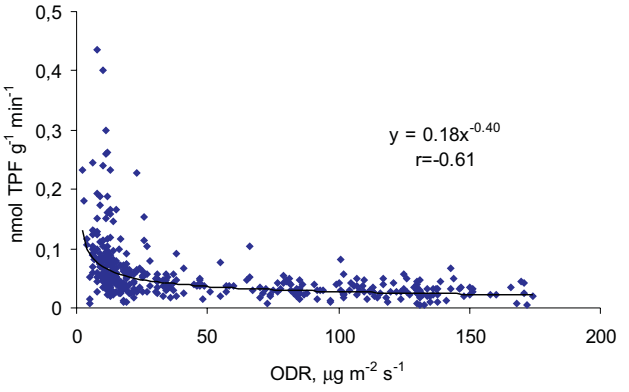

Fig. 5. Relation between soil dehydrogenase activity and oxygen diffusion rate (results of entire experiment included).

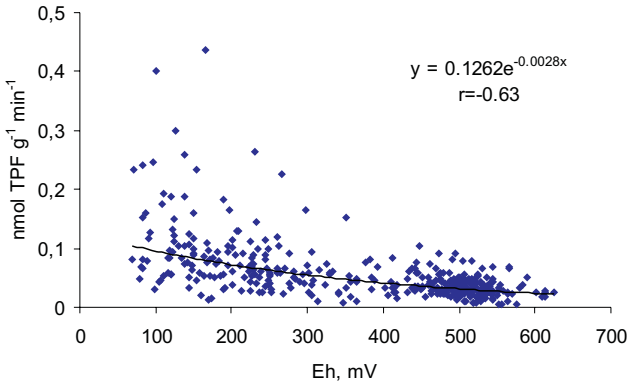

Fig. 6. Dehydrogenase activity in relation to soil redox potential (results of entire experiment included).

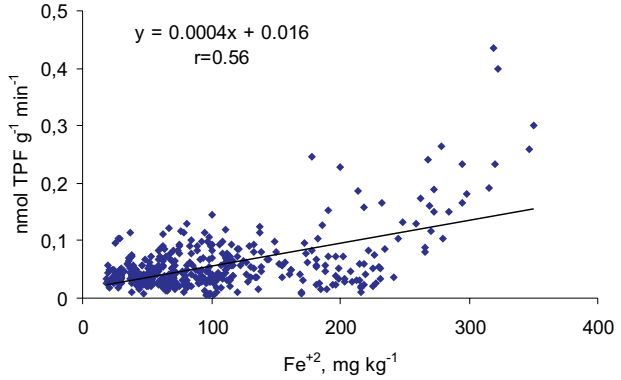

Fig. 7. Relation between soil dehydrogenase activity and Fe^{+2} concentration (results of entire experiment included).

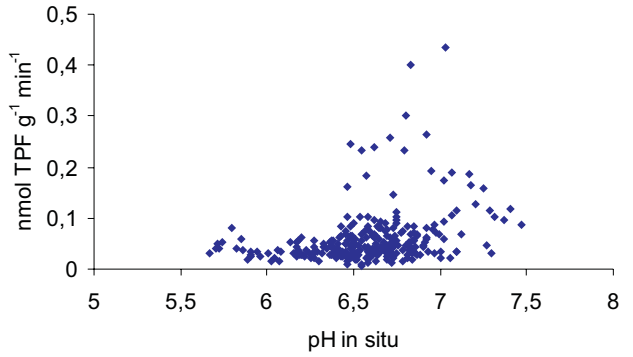

Fig. 8. Dehydrogenase activity in relation to pH in situ, determined by air-water conditions.

observed at the beginning of the stress I. The dehydrogenase activity of that soil was only 0.0048 nmol TPF g^{-1} min^{-1}. By contrast, the maximal dehydrogenase activity (0.436 nmol TPF g^{-1} min^{-1}) was accompanied by the lowest Eg value (only 0.0001 m^3m^{-3}). This is indicative of the fact that the modification of the main physical factors (as well as the additional intensification of the processes by high temperature, see Fig. 1) resulted in a drastic decrease of Eg and as high as a 90-fold increase of dehydrogenase activity. The dehydrogenase activity was negatively correlated with Eg (Fig. 4).

ODR measurements give information about the presence of O_2, available in the soil for plant roots whereas Eh is concerned with all redox transformations, resulting from the actual composition of the soil gas phase and soil solution [20]. Dehydrogenase activity increased with the decrease of ODR below 30 μg m^{-2} s^{-1} (Fig. 5) and that of Eh below 300 mV (Fig. 6). One of the aspects of flooded soil reduction in the absence of oxygen is the increase in the content of reduced iron. This is because anaerobic micro-organisms use Fe^{+3} iron as terminal electron acceptors in their respiration pathways. Dehydrogenase activity was positively related to reduced Fe concentration (Fig. 7).

Figure 8 shows the natural alteration of pH in situ and its relation to soil dehydrogenase activity during barley vegetation under different air-water conditions. Soil pH changed from 5.5 to 7.5. The direction of these changes was determined by anaerobic transformations. The maximum enzyme activity was observed at a soil pH of 6.6-7.2.

The results from the pot experiment with barley vegetation are consistent with the results obtained in the laboratory without plants [2,10,12,21]. Dehydrogenase activity is a sensitive biochemical indicator of soil aeration. The level of the activity reflects the metabolic status of microorganisms in the soil system and is closely correlated with physical and chemical aeration indexes.

CONCLUSIONS

It has been shown that:

1. Changes of dehydrogenase activity of water saturated soil were most intensive at the shooting stage of the barley (stress II). The activity increased significantly between 3rd and 14th day of stress at maximal, for the vegetation season, temperature (28°C).

2. Increases of water content and soil bulk density resulted in a significant increase in dehydrogenase activity ($P<0.001$).

3. Dehydrogenase activity is negatively correlated with Eg, ODR and Eh, and positively with Fe^{+2} content.

4. Maximum dehydrogenase activity was observed at pH in situ of 6.6-7.2. The range of pH in soil with barley vegetation under different aeration conditions was from 5.5 to 7.5.

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