

## Aeration properties and enzyme activity on the example of Arenic Chernozem (Tišice)

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**A b s t r a c t.** The purpose of this paper is to characterise aeration properties of 3 horizons of Arenic Chernozem profiles situated in Tišice (typical for the agricultural production region of Central Bohemia, Czech-Republic) on the basis of results obtained in the multilateral Austrian-Czech-Hungarian-Polish-Slovak project on the "Assessment of Structure in Agricultural Soils" sponsored by the Austrian Ministry of Science and Research. The paper comprises results of measurements of different soil aeration properties such as: oxygen diffusion rate (ODR), air-filled porosity (Eg), relative gas diffusion coefficient (D/D<sub>0</sub>), air permeability (k), and redox potential (Eh) as well as dehydrogenase and catalase activity. Undisturbed soil cores (from a depth of 5, 20 and 40 cm) were tested after equilibration on kaolin tension plates with soil water tensions 0 (capillary saturation – pF 0), 63 (pF 1.8), 159 (pF 2.2) and 500 hPa (pF 2.7). A significant correlation between the tested parameters was found.

**K e y w o r d s:** aeration properties, enzyme activity, Arenic Chernozem

### INTRODUCTION

Soil enzymes are useful in evaluating the level of biological fertility and, since they are very easy to assay, they are useful indicators in soil microbial studies. Dehydrogenases and catalases belong to the group of oxidoreductases and may both be considered as indicators of microbial oxidative activity in soil.

Dehydrogenase activity in soils provides correlative information on biological activity and microbial populations in soil. Dehydrogenases conduct a broad range of oxidative activities that are responsible for decomposition, i.e., dehydrogenation, of organic matter. They represent a class of enzymes that gives us information about the influence of natural environmental conditions on biological activity (Schäffer, 1993).

Since catalase is found in all aerobic microorganisms except for obligatory anaerobes, its activity can inform us about soil aeration status.

Physical conditions of the soil, e.g., water content and aeration influence the microbial populations and their viability. The effect of soil aeration status on the enzymatic activities can help to understand nutrients transformation in the soil.

The purpose of this paper is to characterise aeration status of one Czech soil on the basis of results obtained in the multilateral Austrian-Czech-Hungarian-Polish-Slovak project on the 'Assessment of Structure in Agricultural Soils' sponsored by the Austrian Ministry of Science and Research. The measurements comprised oxygen diffusion rate (ODR), relative gas diffusion (D/D<sub>0</sub>), air permeability (k), redox potential (Eh) as well as dehydrogenase and catalase activities.

### MATERIALS AND METHODS

#### Soil

The experiments were carried out on the soil from Central Bohemia situated in a typical region for agricultural production. The soil profiles are situated at Tišice (district Mělník, about 20 km north-west Prague), on a quaternary fluvial terrace of gravel sand, overlaid by sand. The landscape is practically plain. The whole experimental area is occupied by the Arenic Chernozems of the carbonate variety (according to FAO-classification system). The undisturbed soil samples from 3 soil pits (T1, T2, T3) were collected from three soil horizons - Ap (0-30) - 5 cm, Ap (0-30) - 20 cm and A/Ck (30-50) - 40 cm.

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Their basic properties are presented in Table 1 and full description and characteristics in the paper by Gliński (1993).

ment, a device described by Malicki and Walczak (1983), with an automatic control of the effective reduction voltage was used. Four platinum wire electrodes (0.5 mm × 4 mm)

**Table 1.** Basic properties of the studied soil

Profile	Horizon - depth (cm)	Particle size distribution ( $\mu\text{m}$ )			Bulk density ( $\text{Mg m}^{-3}$ )	pH		O.M. (%)
		2000-50	50-2	<2		H <sub>2</sub> O	KCl	
T1	Ap (0-30) -5	63	23	14	1.59	8.8	7.9	3.6
T2	Ap (0-30) - 20	59	23	18	1.64	8.3	7.4	3.6
T3	A/Ck (30-50) - 40	47	37	16	1.24	9.0	8.3	1.2

### Measurement methods

Undisturbed soil samples in 100 cm<sup>3</sup> brass cylinders were collected in late autumn, 1991 and transported to Lublin in January 92. The measurements of all the above mentioned parameters were made at soil moisture tension of: 0 hPa (capillary saturation), 63 hPa (pF 1.8), 159 hPa (pF 2.2) and 500 hPa (pF 2.7). Undisturbed soil cores representing each horizon after capillary saturation were equilibrated with particular soil moisture tensions on kaolin tension plates. At each equilibrium, a relative gas diffusion coefficient ( $D/D_0$ ) and air permeability ( $k$ ) were measured. When the measurements were completed, the cylinders were re-saturated and, after subsequent equilibration with the consecutive tension plates, were used to determine oxygen diffusion rate (ODR), redox potential (Eh), and the activity of dehydrogenases and catalases.

The measurement of  $D/D_0$  was performed according to the unsteady-state method of Stepniewski (1983) with the modification of the sample holder described by Stepniewski (1981) using oxygen as a diffusing agent. The method is also described by Gliński and Konstankiewicz (1991). The soil core in this method is situated horizontally. Non-shrinking cores in this device are held in the cylinder, but shrinking cores (if they are stable enough) can also be installed after removing them from the cylinder.

The measurement of air permeability ( $k$ ) was performed at 10 hPa air pressure with a laboratory permeameter type LPIR-1 produced by the Experimental Department of Metallurgy in Cracow. The soil core (in the cylinder) was placed vertically in the device and air was blown through it from the bottom (Gliński and Stepniewski 1985, Ball *et al.* 1981).

The oxygen diffusion rate (ODR) method consists in amperometric measurements of electric current intensity corresponding to oxygen reduction on a platinum cathode placed in the soil and negatively polarised with respect to the reference electrode. The indicator is a measure of potential oxygen availability for plant roots. For the ODR measure-

were placed at a depth of 2 cm and polarised to -0.65 V versus saturated calomel electrode, during 4 min. The principle of the method is described in detail by Gliński and Stepniewski (1985) and Gliński and Konstankiewicz (1991).

Redox potential (Eh) was measured potentiometrically using four Pt electrodes (of the same type as for ODR) saturated calomel electrode as the reference electrode, and a laboratory pH-meter (Radiometer, Copenhagen). The electrodes were placed at a depth of 2 cm. The measurements were taken after stabilisation of the readings (Gliński and Stepniewski, 1985).

Dehydrogenase activity was measured by the method of TTC (2,3,5-triphenyltetrazolium chloride) reduction to formazan during incubation for 20 h at 30°C, at pH=8.2 according to procedure of Casida *et al.* (1964).

Catalase activity was measured by manganometric titration of surplus of H<sub>2</sub>O<sub>2</sub> under acidic conditions according to the procedure by Johnson and Temple (1964).

Water content, bulk density and particle density were determined by the methods according to Turski *et al.* (1983).

All analytical results were calculated on the basis of oven-dry (105°C) soil mass.

Observations of the correlation between the investigated soil parameters in all horizons, subjected to pre-incubation at controlled water content, were confirmed by statistical analysis of variance and regression of data (Tables 2-4). The linear ( $Y=a+bx$ ), exponential ( $Y=\exp(a+bx)$ ), multiplicative ( $Y=ax^b$ ) and reciprocal ( $1/Y=a+bx$ ) models were used for the description of the analysed relations.

### RESULTS AND DISCUSSION

Pre-incubation of the soil material under various moisture conditions differentiated the physical, physico-chemical and biochemical parameters of the investigated soils. Three horizons of the Arenic Chernozem profiles were investigated.

A decrease of moisture content following changes in the soil moisture tension from 0 to 500 hPa caused changes of the soil aeration status towards oxic conditions. This

**Table 2.** Significance of the effect of sampling depth and of soil moisture tension (s.m.) on the individual studied particular parameter

Factor/ parameter	Eg	k	D/D <sub>0</sub>	ODR	Eh	Dehydro- genase	Catalase
Depth	n.s.	n.s.	n.s.	n.s.	+	+	+
s.m. tension	+	+	+	+	n.s.	n.s.	n.s.

Explanation: + indicates significant differences, n.s. - no significant differences.

**Table 3.** Statistically significant differences in the tested parameters between individual soil moisture tension of the Arenic Chernozem horizons

Soil moisture tension (contrast)	Eg	k	D/D <sub>0</sub>	ODR	Eh	Dehydro- genase	Catalase
0 - 63	+	+	n.s.	n.s.	n.s.	n.s.	n.s.
0 - 159	+	+	+	+	n.s.	n.s.	n.s.
0 - 500	+	+	+	+	n.s.	n.s.	n.s.
63 - 159	n.s.	n.s.	n.s.	+	n.s.	n.s.	n.s.
63 - 500	+	+	+	+	n.s.	n.s.	n.s.
159 - 500	n.s.	n.s.	n.s.	+	n.s.	n.s.	n.s.

Explanation: + denotes a statistically significant increase, with tension increase; n.s. - no significant differentiation.

**Table 4.** Correlation between aeration indicators calculated for all soil water tensions of all the horizons treated together

Parameter	D/D <sub>0</sub>	ODR	Eg	Water content by mass
k	0.92*** y = a + bx	0.81*** y = a + bx	0.72** y = a + bx	-0.60* y = a + bx
D/D <sub>0</sub>		0.79** y = a + bx	0.59* y = a + bx	n.s.
ODR			0.79** y = e <sup>(a+bx)</sup>	-0.70* y = e <sup>(a+bx)</sup>
Eg				-0.97*** y = a + bx

\* - significant at P < 0.05, \*\* - significant at P < 0.01, \*\*\* - significant at P < 0.001, n.s. - no significant differentiation.

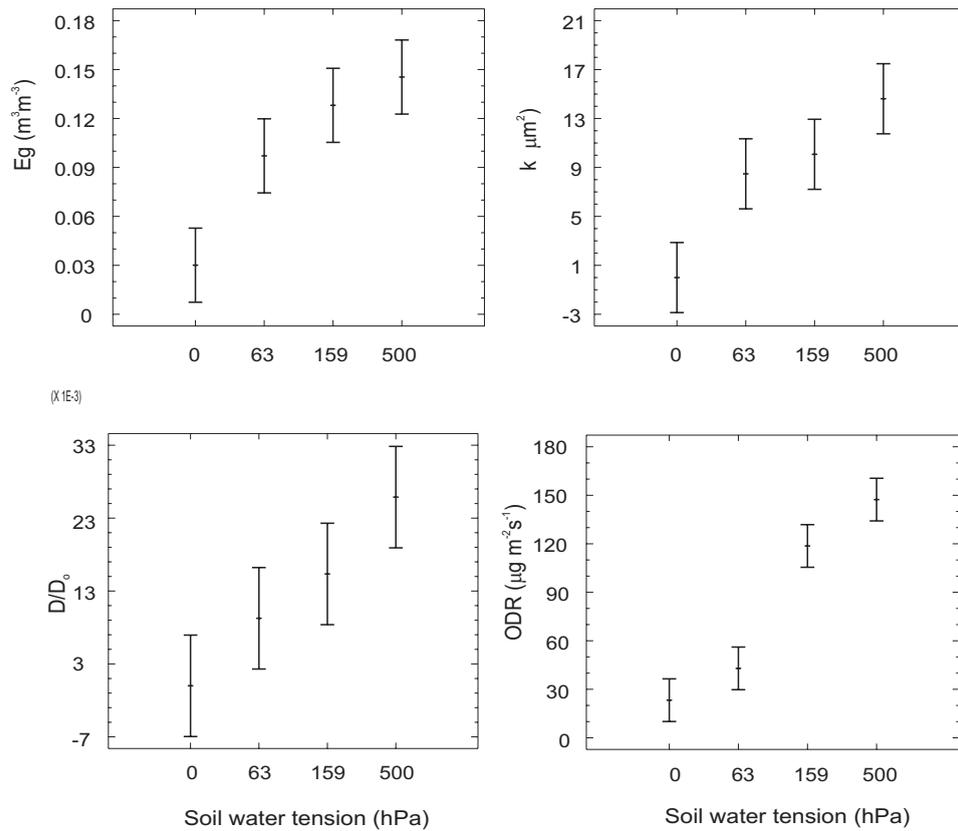
tendency was confirmed by a significant increase of soil oxygenation parameters like: Eg, k, D/D<sub>0</sub> and ODR (Fig. 1), but there was no significant differentiation in the dehydrogenase and catalase activity or Eh value (Tables 2 and 3).

Changes of oxygenation parameters – Eg, k, D/D<sub>0</sub>, ODR and Eh in particular soil horizons of the studied soil are shown in Figs 2-6.

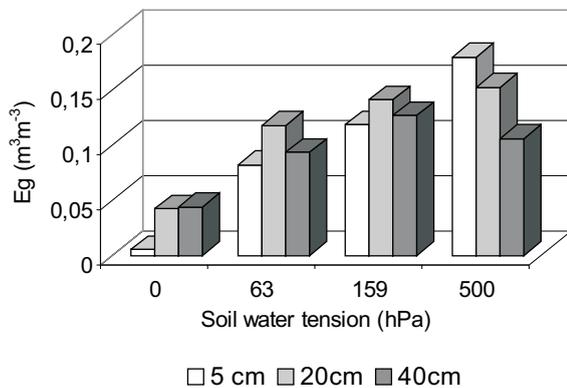
The air filled porosity (Eg) was deeper in higher horizons and increased monotonically with respect to soil moisture tension in the range of 0-500 hPa. The average values of Eg were 0.030, 0.097, 0.128, 0.145 m<sup>3</sup> m<sup>-3</sup> at 0, 63, 159 and hPa, respectively (Fig. 2). It is reasonable to assume the air content of above 0.25 m<sup>3</sup> m<sup>-3</sup> to be sufficient for good aeration. In the range of air content from 0.10 to 0.25 m<sup>3</sup> m<sup>-3</sup>,

aeration may be deficient under some conditions while the air contents below 0.10 m<sup>3</sup> m<sup>-3</sup> characterised decidedly deficient aeration (Gliński and Stępniewski, 1985). This indicated that the investigated soil displayed decidedly deficient aeration conditions at 0 and 63 hPa and aeration may be deficient at 159 and 500 hPa. Analysis of variance did not show any significant differences between the horizons (Table 2) but differences between soil moisture tension levels were found (Table 3). Air-filled porosity (Eg) showed an obvious negative correlation with water content by weight (Table 4).

Air permeability for the studied horizons slowly increased with soil moisture tension (Fig. 3). In all the horizons saturated with water (0 hPa) the k values were equal



**Fig. 1.** Statistically significant differences in the tested parameters ( $E_g$ ,  $k$ ,  $D/D_0$  and ODR) between individual soil water tension levels.

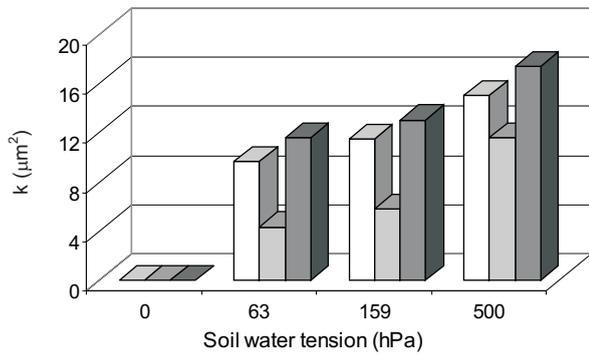


**Fig. 2.** Air-filled porosity ( $E_g$ ) of the three horizons of Arenic Chernozem incubated at particular soil water tension levels.

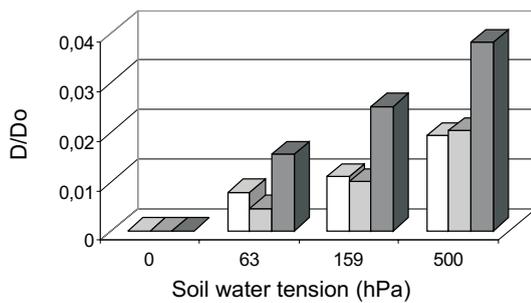
zero. The  $k$  values were 8.48, 10.08 and 14.61  $\mu\text{m}^2$  at 63, 159 and 500 hPa, respectively. Analysis of variance did not show significant differences between the horizons (Table 2), while such differences between 0 hPa and remaining soil moisture tensions were observed (Table 3). Air permeability ( $k$ ) was positively correlated with  $D/D_0$ , ODR,  $E_g$  and negatively correlated with water content for all the horizons taken together (Table 4).

A supplementary indicator of the soil oxygenation status is relative gas diffusion coefficient ( $D/D_0$ ). This parameter showed a monotonic change in the range of 0-500 hPa. A relative gas diffusion coefficient was higher in deeper horizons and increased with an increasing soil water tension (Fig. 4). In all the horizons saturated with water (0 hPa), the  $D/D_0$  values were equal to zero. The average values of  $D/D_0$  were 0, 0.0093, 0.0154 and 0.0259 at 63, 159 and 500 hPa, respectively. Literature quote  $D/D_0 = 0.005$  as a lower critical value corresponding to low respiration activities, and  $D/D_0 = 0.02$  as the upper value for the highest respiration rates (Gliński and Stepniewski, 1985). Relating these threshold values to the studied soil, we can confirm that there were favourable conditions for gas exchange at the highest respiration rates only at the soil moisture tension of 500 hPa. For low respiration rates, gas exchange is sufficient already at 63 hPa. Analysis of variance did not show any significant differences between the horizons (Table 2) but such differences between soil moisture tensions were found (Table 3). The relative gas diffusion ( $D/D_0$ ) was positively correlated with ODR and  $E_g$  for the three horizons considered together (Table 4).

All three horizons studied showed a relatively high level of oxygen diffusion rate (Fig. 5). A typical tendency towards



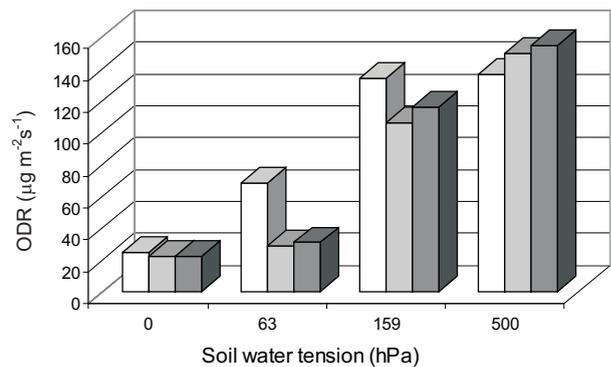
**Fig. 3.** Air permeability ( $k$ ) of the three horizons of Arenic Chernozem incubated at particular soil water tension levels. Explanations as in Fig. 2.



**Fig. 4.** Relative gas diffusion coefficient ( $D/D_0$ ) of the three horizons of Arenic Chernozem incubated at particular soil water tension levels. Explanations as in Fig. 2.

This suggests high soil redox resistance, related to numerous soil properties (Gliński and Stepniewska, 1986). None of the soil water tension levels reached Eh below 400 mV corresponding to the initiation of nitrate reduction (Stepniewska, 1988). The reason for high levels of Eh may be the presence of nitrates. It has been found (Bailey and Beauchamp, 1971; Gliński *et al.*, 1990; Gliński *et al.*, 1991), that soil amendment with nitrates maintains soil redox potential for a certain period constant and delays reduction of Mn (IV) and Fe (III) compounds. Analysis of variance showed significant differences between the horizons (Fig. 7) and lack of significant differences between particular soil moisture tension levels (Tables 2 and 3).

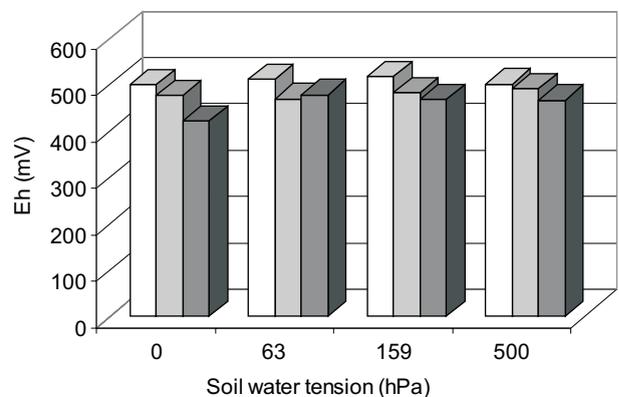
Dehydrogenase activity varied widely in the tested horizons (Fig. 8). The enzyme activity decreased with depth and with the increase of the soil moisture tension. The highest dehydrogenase activity (0.0140 nmol formazan



**Fig. 5.** Oxygen diffusion rate (ODR) of the three horizons of Arenic Chernozem incubated at particular soil water tension levels. Explanations as in Fig. 2.

the ODR increase with increasing soil moisture tension levels was observed in all the horizons. The values of ODR showed an abrupt increase in the range of soil water tension of 63-169 hPa. The average value of ODR at 0 hPa ( $23.3 \mu\text{g m}^{-2}\text{s}^{-1}$ ) differed significantly from the value at 63 hPa ( $42.9 \mu\text{g m}^{-2}\text{s}^{-1}$ ), at 159 hPa ( $118.7 \mu\text{g m}^{-2}\text{s}^{-1}$ ) and at 500 hPa ( $147.3 \mu\text{g m}^{-2}\text{s}^{-1}$ ). The critical ODR value, usually considered to be below  $30 \text{ g m}^{-2}\text{s}^{-1}$ , can be expected only at the soil water tension of 0 hPa in the all horizons and below 63 hPa in the deeper horizons. The analysis of variance did not show any significant differences between the horizons (Table 2) but significant differences between soil moisture tensions were observed (Table 3). Oxygen diffusion rate (ODR) was positively correlated with Eg and negatively correlated with water content in all the horizons taken together (Table 4).

The investigated soil was characterised by high redox potential values, which never dropped below 400 mV irrespective of soil water tension in any soil horizons (Fig. 6).

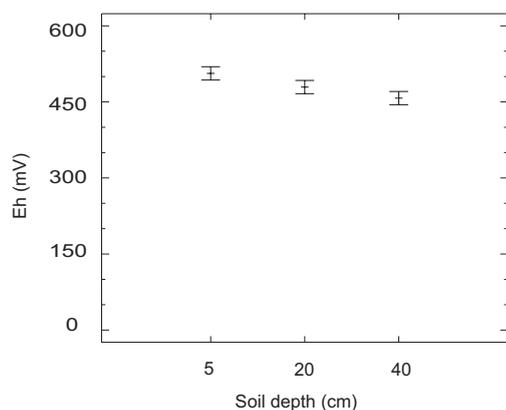


**Fig. 6.** Redox potential (Eh) of the three horizons of Arenic Chernozem incubated at particular soil water tension levels. Explanations as in Fig. 2.

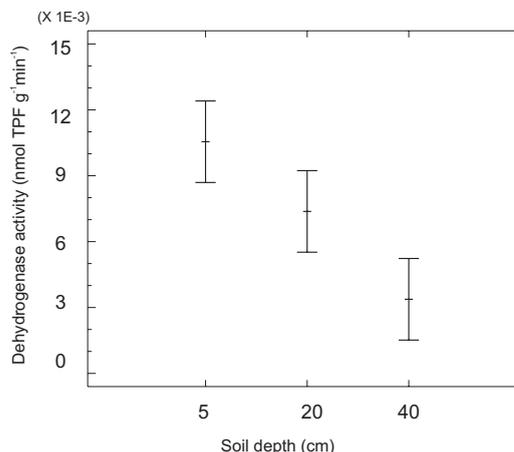
$\text{g}^{-1}\text{min}^{-1}$ ) occurred in the surface horizon pre-incubated at full saturation with water, and the lowest ( $0.0028 \text{ nmol formazan g}^{-1}\text{min}^{-1}$ ) - in A/Ck horizon, after pre-incubation at the soil moisture tension of 500 hPa. The second horizon exhibited dehydrogenase activity from  $0.0082 \text{ nmol formazan g}^{-1}\text{min}^{-1}$  in the soil pre-incubated at full saturation with water to  $0.0065 \text{ nmol formazan g}^{-1}\text{min}^{-1}$  after pre-incubation at the soil moisture tension of 500 hPa. The third horizon showed dehydrogenase activity from  $0.0035$  to  $0.0028 \text{ nmol formazan g}^{-1}\text{min}^{-1}$  in the material pre-incubated at the soil moisture tension of 0 and 500 hPa, respectively. The influence of soil moisture tension on dehydrogenase activity was lower in the deeper horizons as compared to the upper one. Analysis of variance showed statistically significant differences only between the deepest horizon and the two others (Fig. 9) and no significant differences between soil moisture tension levels were found

(Tables 2 and 3). Dehydrogenase activity was correlated positively with  $D/D_0$  ( $r=0.65^*$ ) (Fig. 10). Irrespective of the soil horizon, dehydrogenase activity in general showed a decreasing tendency with a decrease in soil moisture content, but it was not statistically significant. The highest activity was observed in the water saturated material and the lowest in the material pre-incubated at the soil moisture tension of 500 hPa. The range of the average (all horizons) values of enzyme activity was from  $0.00483 \text{ nmol formazan g}^{-1}\text{min}^{-1}$  (for 500 hPa) to  $0.00857 \text{ nmol formazan g}^{-1}\text{min}^{-1}$  (for 0 hPa). The phenomenon of changes in dehydrogenase activity, relating to the soil aeration status had been observed earlier (Gliński *et al.*, 1983; Gliński *et al.*, 1986; Pedrazzini and McKee, 1984; Stepniewski *et al.*, 1993; Brzezińska *et al.*, 1998).

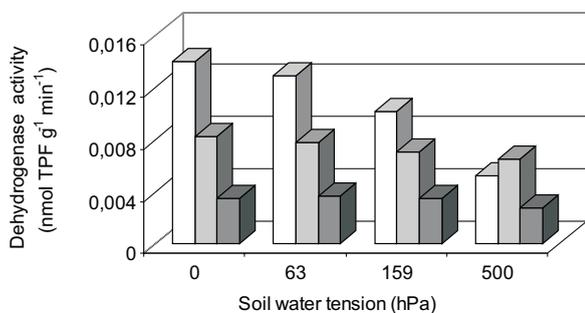
Catalase activity varied widely in the tested horizons (Fig. 11). The highest catalase activity ( $73.9 \mu\text{mol KMnO}_4$



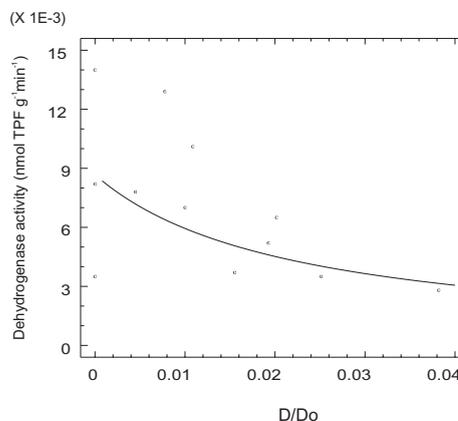
**Fig. 7.** Statistically significant differences in redox potential (Eh) between the three horizons of Arenic Chernozem.



**Fig. 9.** Statistically significant differences in dehydrogenase activity between the three horizons of Arenic Chernozem.

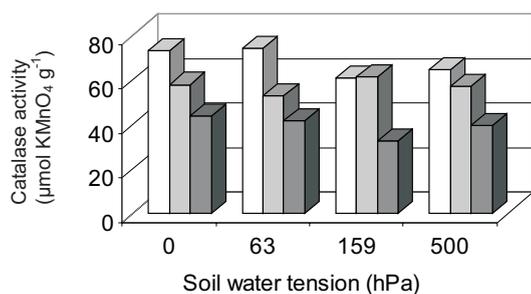


**Fig. 8.** Dehydrogenase activity of the three horizons of Arenic Chernozem incubated at particular soil water tension levels. Explanations as in Fig. 2.

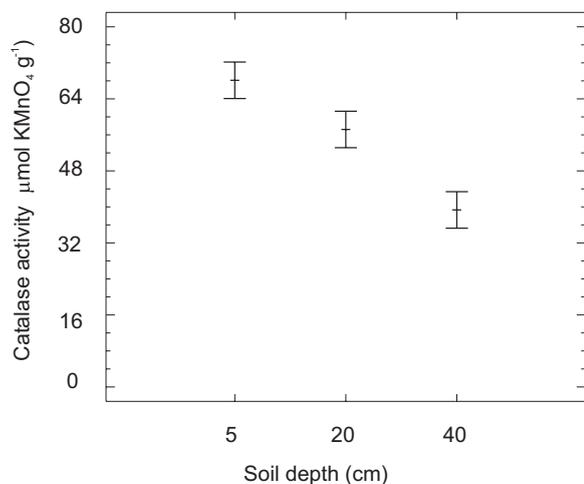


**Fig. 10.** Correlation between dehydrogenase activity versus  $D/D_0$  for the three horizons and all soil water tension levels.

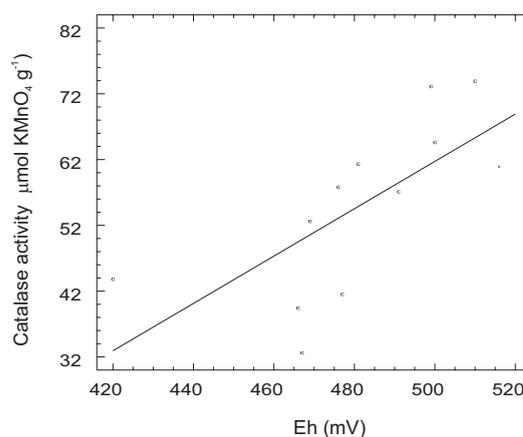
$\text{g}^{-1}$ ) was found in the surface horizons pre-incubated at the soil moisture tension of 0 and 63 hPa, and the lowest ( $32.6 \mu\text{mol KMnO}_4 \text{g}^{-1}$ ) - in the third A/Ck horizon, after pre-incubation at the soil moisture tension of 159 hPa. The second horizon showed a little differentiation in enzyme activity relating to moisture tension. The third horizon showed catalase activity from 43.8 to  $32.6 \mu\text{mol KMnO}_4 \text{g}^{-1}$  in the treatment pre-incubated at soil moisture tension of 0 and 159 hPa, respectively. The range of average (all horizons) values of enzyme activity was from  $53.7 \mu\text{mol KMnO}_4 \text{g}^{-1}$  (for 500 hPa) to  $56.9 \mu\text{mol KMnO}_4 \text{g}^{-1}$  (for 0 hPa). The influence of soil moisture tension on catalase activity was lower in the deeper horizons as compared to the top horizon. The enzyme activity decreased with soil depth. Analysis of variance showed significant differences among the horizons (Fig. 12) and no significant differences between particular soil moisture tension levels were found (Tables 2 and 3). Catalase activity was positively correlated with Eh ( $r = 0.70^*$ ) (Fig. 13).



**Fig. 11.** Catalase activity of the three horizons of Arenic Chernozem incubated at particular soil water tension levels. Explanations as in Fig. 2.



**Fig. 12.** Statistically significant differences in catalase activity between the three horizons of Arenic Chernozem.



**Fig. 13.** Correlation between catalase activity versus redox potential (Eh) for the three horizons and all soil water tension levels.

## CONCLUSIONS

Characteristics of properties that related to aeration in particular soil horizons of the Arenic Chernozem profiles demonstrated their differentiation with soil depth and water tension level. In particular it was found out that:

1. Air-filled porosity increased from  $0.005 \text{ m}^3 \text{ m}^{-3}$  (volume of the entrapped air) at full water saturation in the upper horizons to about  $0.18 \text{ m}^3 \text{ m}^{-3}$  at 500 hPa.
2. Air permeability, was the lowest in the deepest horizon.
3. Gas diffusion ( $D/D_0$ ) unlike  $k$ , was the best in the deepest soil horizon; restricted gas diffusion in the profile can be expected at the soil water tension  $<80$  hPa.
4. ODR values showed an abrupt increase in the range of soil water tension of 63-159 hPa. The critical values being expected only at soil water tension  $<60$  hPa.
5. All aeration parameters were interrelated and showed correlation with soil water content (except of  $D/D_0$  and Eh).
6. Dehydrogenase and catalase activity varied widely in the tested horizons and decreased with soil depth. Dehydrogenase activity was correlated positively with  $D/D_0$  and catalase activity was positively correlated with Eh.

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