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SOME OF ASPECTS OF DEHYDROGENASE ACTIVITY IN SOILS

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A b s t r a c t. Effects of sampling time, storage period, and three different methods of storage on dehydrogenase activity of the topsoil of Phaeozem and three Orthic Luvisols were studied. Analysis of seasonal variability showed statistically significant differences in the dehydrogenase activity between four seasons of the year in the Orthic Luvisol developed from silt. Differentiation among the activity of the soil samples taken in different seasons of the year is maintained for the 7 years of storage. The highest dehydrogenase activity of fresh soils was found in the Phaeozem where it was about four times higher than in the Orthic Luvisol developed from silt. That tendency was preserved in all the experiments irrespective the storage time and methods. Air-drying resulted in a considerable decrease of dehydrogenase activity after sample storage for a period from one to two months with a tendency to increase an increasing storage period exceeding about 20 months. The subsequent preincubation of the air-dried soil samples at room temperature showed a more stabile dehydrogenase activity than in the case of other methods of storage. The results suggest that the worst method of storage for the investigated soils is storage at 4 ^{o}C for the time longer than 1 month. The investigations demonstrated that the main factor affecting the levels of dehydrogenase activity is sampling time. It seems that dehydrogenase activity is affected by the environmental properties in the field so strongly that it "keeps" its activity for a long time.

K e y w o r d s: dehydrogenase activity, methods of storage

INTRODUCTION

Active dehydrogenases are considered to exist in soils as integral parts of intact cells and dehydrogenase activities of the soil microflora [9]. They do not accumulate extracellularly in the soil. Dehydrogenase activity in soils provides correlative information on the biological activity and microbial populations in the soil. Biochemical properties of dehydrogenases do not suggest the presence of free, abiotic dehydrogenases in the soil. However, measurements of dehydrogenase activities in the soil, are important for the soil biology. Measurements of dehydrogenase activity represent immediate metabolic activities of the soil microorganisms at the time of the test [22]. Dehydrogenases conduct a broad range of oxidative activities that are responsible for degradation, i.e., dehydrogenation of organic matter.

Accumulation of evidence over the past decade suggests that the activity of several soil enzymes can be used to assess anaerobic metabolism and thus, indirectly, the oxygen or redox level of the soil. Active dehydrogenase can utilise both O_2 and other compounds as terminal electron acceptors, although anaerobic microorganisms produce most dehydrogenases. Dehydrogenase activity, therefore, may reflect changes in the microbial population as well as the soil redox potential (*Eh*) [24]. Brzezińska *et al.*, [1] found that soil dehydrogenase activity is highly correlated with (*Eh*) and with the oxygen diffusion rates (ODR). She suggested that soil water content and temperature influence the dehydrogenase activity indirectly by affecting the soil oxidation-reduction status.

Dehydrogenases represent a class of enzymes that give us information about the influence of natural environmental conditions of the microbial activities of the soil [20]. Dehydrogenase activity

appears to be more related to the metabolic state of microbial population of the soil than to the activity of specific free enzymes acting on a particular substrate [15].

Little information is available on the stability of the soil dehydrogenase activities and on the effect of type and time of storage. Storage of the soils for periods up to several weeks or months was sometimes necessary. The objective of this paper was to determine whether there is a relationship between dehydrogenase activity and time and kind of storage as well as the season of sampling, during long storage. Moreover, whether there is any characteristic enzyme activity connected with the type of soil, independent from the time and kind of storage and the season of sampling.

The effects of sampling season, storage period, and three different methods of storage on dehydrogenase activities of the topsoil of four soil units were studied, because of the scare available information on the stability of soil dehydrogenase activities during long storage.

MATERIALS AND METHODS

Samples of Orthic Luvisol developed from silt formation (sample F) were collected from the depth of 1-20 cm of a cultivated field in Felin in the Lublin region at twelve dates of the year, from October 1991 to September 1992 [25]. Seven measuring sites that differed with regard to drainage requirements, were chosen in the area under drainage, situated in the east-central part of Poland near Łosice in the Kałuża river-basin [5]. Samples of an Orthic Luvisol developed from silty light loams (samples 73,128,148), sand (samples 162,177) and of a Phaeozem developed from heavy loam (samples 186) and Phaeozem developed from silty light loam (samples 66) were collected from the depth of 1-20 cm of the cultivated fields and meadows in the period 1990 - 1992. Two of the soil samples were collected in spring (V.1990, VI. 1992) and one in autumn (X 1991). Dehydrogenase activities of the fresh samples and their moisture content were determined within 24 h after sampling. The remaining subsamples (ca. 2-kg) collected in 1990-1992 were divided into two parts and stored in three different ways:

Storage at 4 ^{o}C: the soils samples with the field moisture content were kept undried at 4 ^{o}C in a refrigerator up to 20 months.

Air-dry storage at room temperature without preincubation. The soil samples were air-dried at room temperature and stored at room temperature for a period of up to 20 months. The samples stored in this way were collected in the period 1990-92 and were stored air-dried until 1999.

Air-dry storage at room temperature with preincubation. The samples stored air-dry as described above were subjected to dehydrogenase activity determination after preincubation. The preincubation of the samples stored air-dry was performed in plastic cylinders 30 cm^3 in volume and 3 cm in diameter. The procedure comprised saturation of the samples with water and leaving them at room temperature for 2 weeks on kaolin plates of the moisture tension 159 hPa (pF 2.2). This time period was found to be sufficient to reach the moisture equilibrium.

The assessment of dehydrogenase activity was performed according to the procedure of Casida *et al.* [3] on the same day for the samples stored according to the three described methods. Simultaneously, soil moisture content was measured.

Basic properties of the investigated soils are presented in Table 1.

RESULTS AND DISCUSSION

Analysis of the seasonal patterns of the Orthic Luvisol developed from silt material showed the first increase of dehydrogenase activity in March, the maximum of activity in September and the lowest activity in winter (Fig. 1). Statistically significant differences in the enzyme activity in four seasons of the year were shown in Table 2.

Seasonal changes in dehydrogenase activity of the soil sampled in May 1990, October 1991 and June 1992 showed from one to three and half times higher dehydrogenase activity in

T a b l e 1. Basic properties of the soils under investigation [5,10]

Sample number	Soil type	Granulometric composition-fraction (%)				Organic matter (%)
		1-0.1	0.1-0.05	0.05-0.02	<0.02	
		(mm)				
66	Phaeozem (silty light loam)	46	10	19	25	2.5
186	Phaeozem (heavy loam)	34	9	24	33	1.6
73	Orthic Luvisol (silty light loam)	47	11	18	24	1.4
128	Orthic Luvisol (silty light loam)	53	13	17	17	1.8
148	Orthic Luvisol (silty light loam)	43	9	24	24	1.9
162	Orthic Luvisol (sand)	55	11	13	21	1.5
177	Orthic Luvisol (sand)	46	13	20	21	1.6
F	Orthic Luvisol (silt)	20	6	42	6	1.5

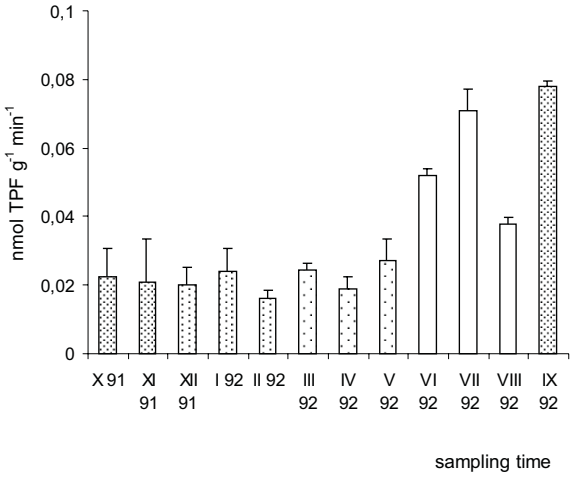

Fig. 1. Effect of the sampling season on dehydrogenase activity of the Orthic Luvisol developed from silt.

T a b l e 2. Statistical significance of differences in dehydrogenase activity related to sampling seasons of the Orthic Luvisol developed from silt. (On the basis of 95% LSD method)

Season of the year (contrast)	Dehydrogenase activity
Spring-autumn	-
Spring-winter	0
Spring-summer	+
Autumn-winter	-
Autumn-summer	0
Winter-summer	+

(-) - Significant decrease with respect to the season listed first, (+) - significant increase with respect to the season listed first, (0) - No significant difference with respect to the season listed first.

May and June than in October for the Orthic Luvisol developed from silty light loam and sand. But in the case of Phaeozem (sample 186) the investigated enzyme was about twice higher in June and October than in May. As a rule, dehydrogenase activity was higher in June than in October (Fig. 2).

Ross and Roberts [17,19] noted that dehydrogenase activities vary with the season and were the highest in the sample taken in August and significantly correlated with both soil moisture content and pH, under New Zealand conditions. Pauli [13] found the maximum dehydrogenase activity under grass in summer,

under South African conditions. Harris [7] found high dehydrogenase activity in the light sand, as well as in the recently wetted soil. Włodarczyk [25] found that in the investigated Orthic Luvisol developed from silt, dehydrogenase activity was five times higher in September than in February. Spring revival of microbial life in the soil caused a slight increase of dehydrogenase activity in March connected with intensification of oxidation-reduction reactions and temperature change. Gliński *et al.* [6] noted a decrease of dehydrogenase activity in June in the meadow soil and its rapid increase in July which was related to simultaneous soil drying and wetting. Similar results were obtained by Cochran *et al*. [4] and Kandeler and Murer [8].

A comparison of dehydrogenase activity observed in four types of fresh soils showed that the highest activity in the Phaeozem was about four times higher than in the Orthic Luvisol developed from silt (Fig. 3). This tendency was maintained during the whole experimental

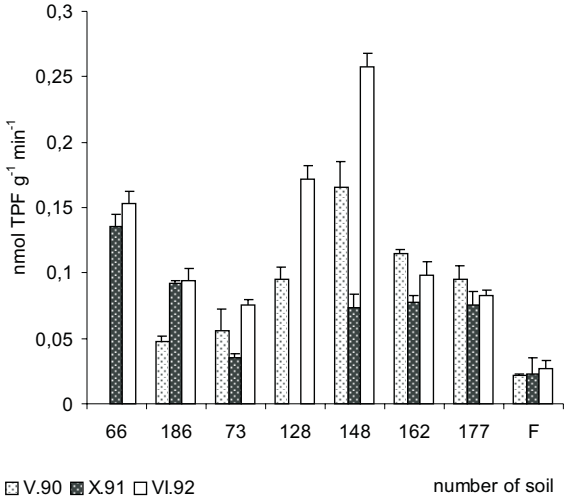

Fig. 2. Dehydrogenase activity of a fresh Phaeozem developed from silty light loam and heavy loam (samples 66,186) and of the Orthic Luvisol developed from silty light loam (samples 73,128,148), sand (samples 162,177) and silt (sample F) sampled in May 1990, in October 1991 and in June 1992.

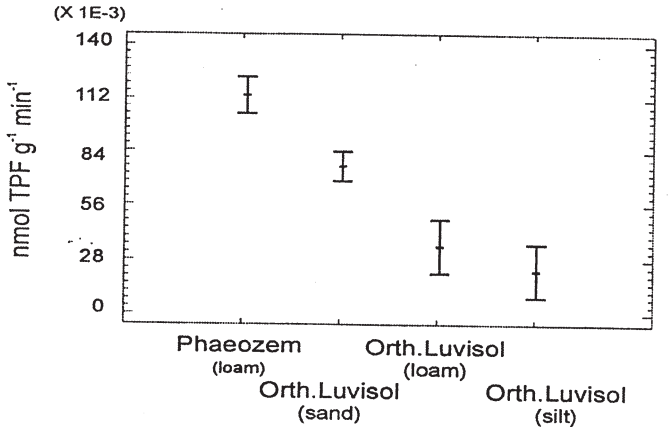

Fig. 3. Average values of dehydrogenase activity of fresh soils sampled in 1991 as a function of the type of soil. The bars represent 95% LSD method.

period independently of the time and method of storage (Fig. 4).

Ross and Roberts [19] found that the levels of enzyme activities may vary broadly influenced by the soil groups and especially by the soil properties.

Each of the organic and mineral fractions in both bulk soil and in the rhizosphere had a special influence on the total enzymatic activity of the examined soils [2,11].

Changes of dehydrogenase activity related to the methods and time of storage are shown in Figs 5 to 8, for the soil samples taken in 1991 and stored for more than 20 months.

Air-drying resulted in a considerable decrease of dehydrogenase activity after sample storage for a period from one to two months with a tendency to increase with the storage period exceeding about 20 months. The most significant decrease was observed in the Phaeozem developed from silty light loam (sample 66) and Phaeozem developed from heavy loam (sample 186), with appreciable increase after four months of storage (Fig. 5). A similar storage trend was observed in the Orthic Luvisols samples developed from silty light loam (samples 73 and 148) (Fig. 6). The remaining Orthic Luvisol samples developed from sand (Fig. 7) or developed from silt (Fig. 8) showed a similar tendency of the dehydrogenase activity formation. Under these conditions storage period of air-dried soils at room temperature for 4 months, after which the dehydrogenase activity significantly dropped should be taken into consideration. Further decrease in the dehydrogenase activity that occurred in the two soils developed from sand was observed. However, in the remaining soils, an increase of the investigated enzyme was observed. At the end of experiment, dehydrogenase activity significantly declined in one of the soils (sample 162) as compared to the fresh soil after its sampling. The presented figures suggest that dehydrogenase activity of the investigated soils, stored at room temperature are related to the type of soil and time of storage.

Ross [15] Pancholy and Rice [12] and Ross and McNeilly [18] proved that dehydrogenase activities decreased when the soils were dried at room temperature. Peterson [14] and Ross [15] found a reduction of dehydrogenase activity when air-dried soil was stored at room temperature, showing a tendency for a decrease with storage time. Casida [3] found that dehydrogenase activity of a cornfield also declined markedly between two to four months of storage at room temperature. Ross [15] found that storage at room temperature may be satisfactory for the

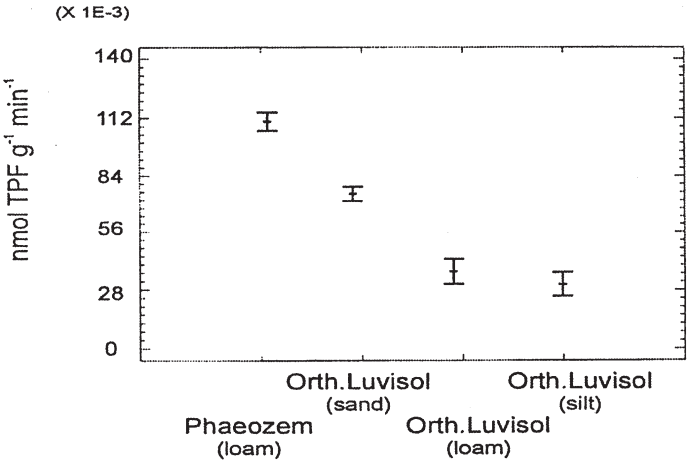

Fig. 4. Average values of dehydrogenase activity of soils sampled in 1991 and those stored up to 20 months (for all methods and periods of storage together) as a function of the type of soil. The bars represent 95% LSD method.

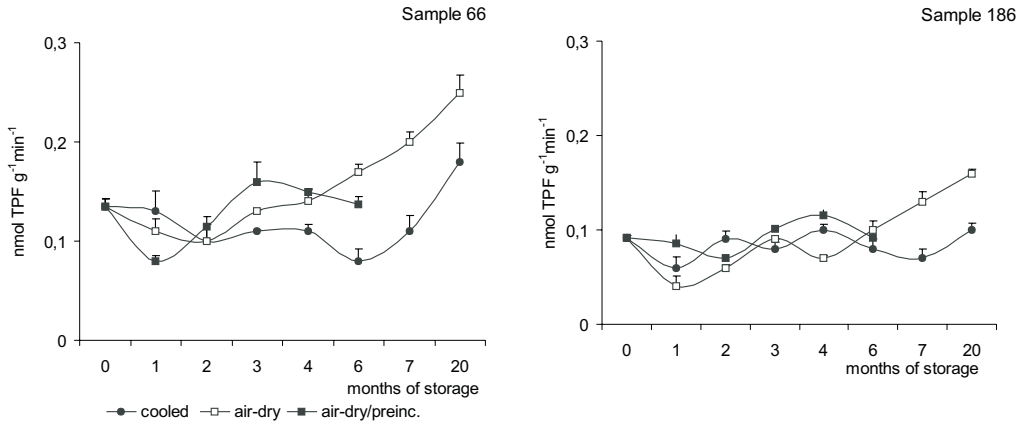

Fig. 5. Influence of the method and storage period on the dehydrogenase activity of the two Phaeozems sampled in October 1991.

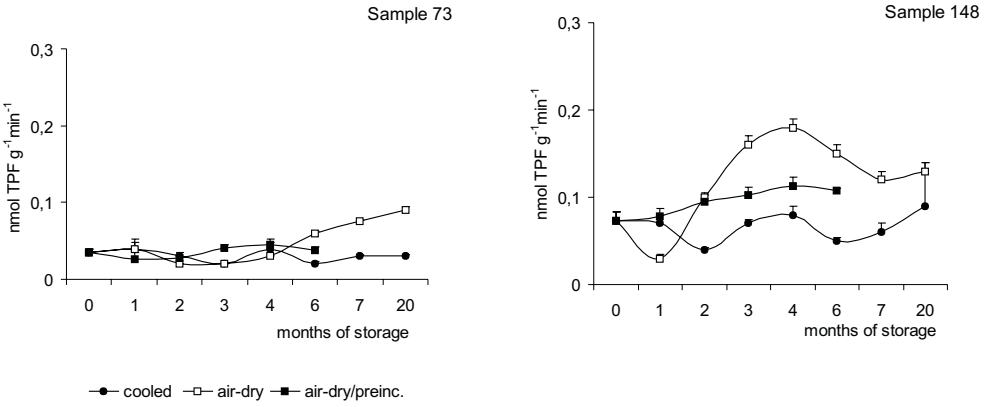

Fig. 6. Influence of the method and storage period on the dehydrogenase activity of the Orthic Luvisols developed from silty light loam sampled in October 1991.

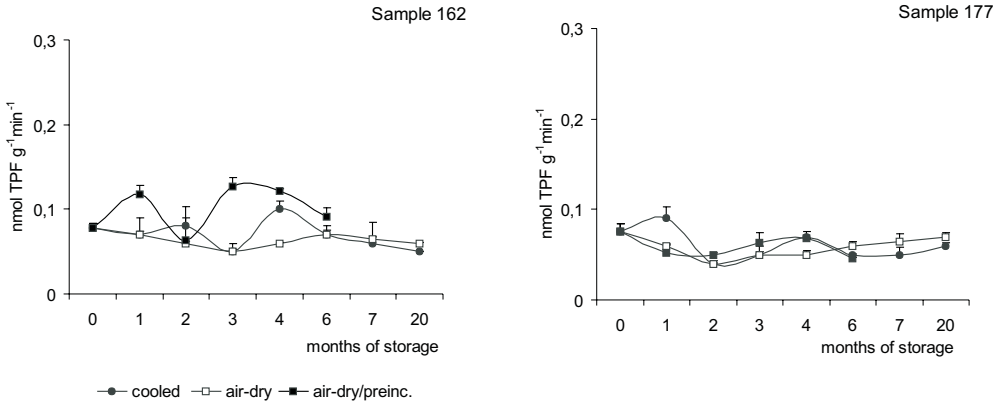

Fig. 7. Influence of the method and storage period on the dehydrogenase activity of the Orthic Luvisol developed from sand sampled in October 1991.

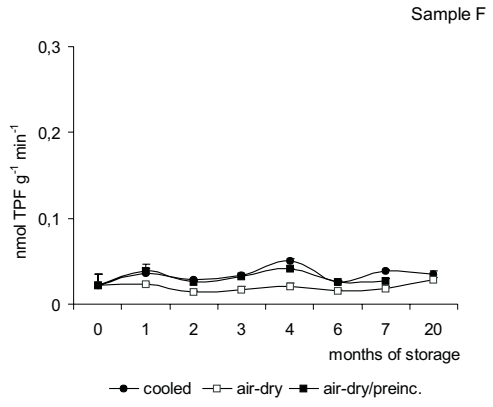

Fig. 8. Influence of the method and storage period on the dehydrogenase activity of the Orthic Luvisol developed from silt sampled in October 1991.

samples of some soils (for example: yellow-grey earth, yellow-brown earth,) for short periods but would be unsuitable for the samples of other soils, e.g., yellow brown loam or podzolized yellow-brown earth.

The aim of preincubation of air-dried soils stored at room temperature was to answer the question whether there is some characteristic dehydrogenase activity of the soil independent of the methods used, sampling time or storage. The soil samples which were air-dried at room temperature and than preincubated showed more stabile dehydrogenase activity than the other methods of storage. Only a slight tendency to increase in relation to the air-dried and fresh soil samples was observed. After 6 months of storage dehydrogenase activity was almost the same in the air-dried and preincubated soil samples except for two cases (samples 66 and 148). The preincubated samples (after air-drying) showed dehydrogenase activities similar to those of the undried (4 ^{o}C) samples in the case of the Orthic Luvisol developed from sand (sample 177). Slightly lower dehydrogenase activity in the preincubated soils as compared to the undried (4 ^{o}C) samples was found in the Orthic Luvisol developed from silt (sample F) and insignificantly higher than the rest of the exa- mined samples. Six months of storage of soil samples under air-dry conditions and their sub- sequent preincubation showed that there is no evidence to prove that there are some characteristic dehydrogenase activity of the soil independent of the time and method of storage and season of sampling of the investigated soils. The same results were obtained by Włodarczyk [25] who studied Orthic Luvisol developed from silt, sampled in October 1991.

Dehydrogenase activity of the undried soil samples kept at 4 ^{o}C (cooled) for about one month was similar to the activity in the fresh samples. Their significant decline after the above time of storage was observed. Figures 5-8 show that the storage behaviour of the soil samples at 4 ^{o}C differed considerably after the first months in the samples developed from different soil material. In the Phaeozem dehydrogenase activity measured after 7 months of storage increased significantly as compared to this activity at the beginning of the experiment, in spite of the decrease in the moisture content observed in the soil samples. Most of the studied soils after 20 months of storage at 4 ^{o}C showed a similar level of dehydrogenase activity and moisture content as the air-dried samples.

Rice [12], Peterson [14], Ross [15] and Pancholy found that dehydrogenase activity was reduced when the soil was stored at 4 ^{o}C and this decrease was generally reduced at -20 ^{o}C [15,16,18]. The discussed reduction tended to increase with the length of storage [12] and the minimum drop of activity occurred at 4 ^{o}C [14,15]. Pancholy and Rice [12] and Peterson [14] reported a slight increase in the dehydrogenase activity in the moist soil during the first few days of storage at room temperature, or in a refrigerator. However, dehydrogenase activity then declined. Casida *et al*. [3] noted a gradual decrease in the dehydrogenase activity of the moist soil kept at room temperature.

A statistical significance of the differences in dehydrogenase activity levels found at different methods of storage and time of sampling of the Phaeozem developed from silty light loam and heavy loam as well as of the Orthic Luvisol developed from silty light loam,

sand and silt (on the basis of 95% LSD method) are shown in Table 3 and Fig. 9. Differences were the highest in the freshly taken soil samples and cooled soils as well as air-dry and cooled soil samples, in the entire period of storage. In most of the analysed soils the most distinct drop of dehydrogenase activity took place undried soil samples stored at 4 ^{o}C. The fewest differences were noted between the fresh soil samples and air-dried and also between air-dried and subsequently preincubated. Our results indicate that the worst method of storage for the investigated soils is storage at 4 ^{o}C for the time period longer than 1 month. It was shown that the change of moisture content affected the level of dehydrogenase activity and its stability. Ross [15] found the greatest differences between the air-dried and undried stored soil samples and suggested that dehydrogenase activity of the soil and its stability during storage at 4 ^{o}C was lower in the samples of lower moisture content.

Our results show that the storage behaviour of the studied soils depended on the type of soil and its sampling time. The least sensitive soil in regard to type of storage was the Orthic Luvisol developed from sand, followed by the Phaeozem developed from heavy loam and silty light loam, and as the last in the series was - the Orthic Luvisol developed from silt and silty light loam. Dehydrogenase activity appears to be dependent on the time of soil sampling. Table 3 shows different behaviour of the same type of soil under the same method of storage depending on the month and year of soil sampling.

According to the results obtained here, the main factor affecting the levels of dehydrogenase activity of the soil is sampling time.

T a b l e 3. Statistical significance of differences in dehydrogenase activity among different methods of storage and sampling time (on the basis of 95% LSD method)

Method of storage	Type of soil			
	Phaeozem (loam)	Orthic Luvisol (loam)	Orthic Luvisol (sand)	Orthic Luvisol (silt)
		05.1990		
Fresh - air-dry	+	-	-	0
Fresh - cooled	+	-	-	0
Fresh - air-dry/preinc.	0	-	-	0
Air-dry - air-dry/preinc.	0	-	0	0
Air-dry - cooled	0	-	0	0
Cooled - air-dry/preinc.	0	0	0	0
		10.1991		
Fresh - air-dry	0	0	0	0
Fresh - cooled	0	0	0	+
Fresh - air-dry/preinc.	0	0	0	+
Air-dry - air-dry/preinc.	0	0	0	+
Air-dry - cooled	-	0	0	+
Cooled - air-dry/preinc.	0	0	+	-
		06.1992		
Fresh - air-dry	0	0	0	0
Fresh - cooled	-	-	0	-
Air-dry - cooled	-	-	0	-

(-) - Significant decrease with respect to the method listed first, (+) - significant increase with respect to the method listed first, (0) - No significant difference with respect to the method listed first.

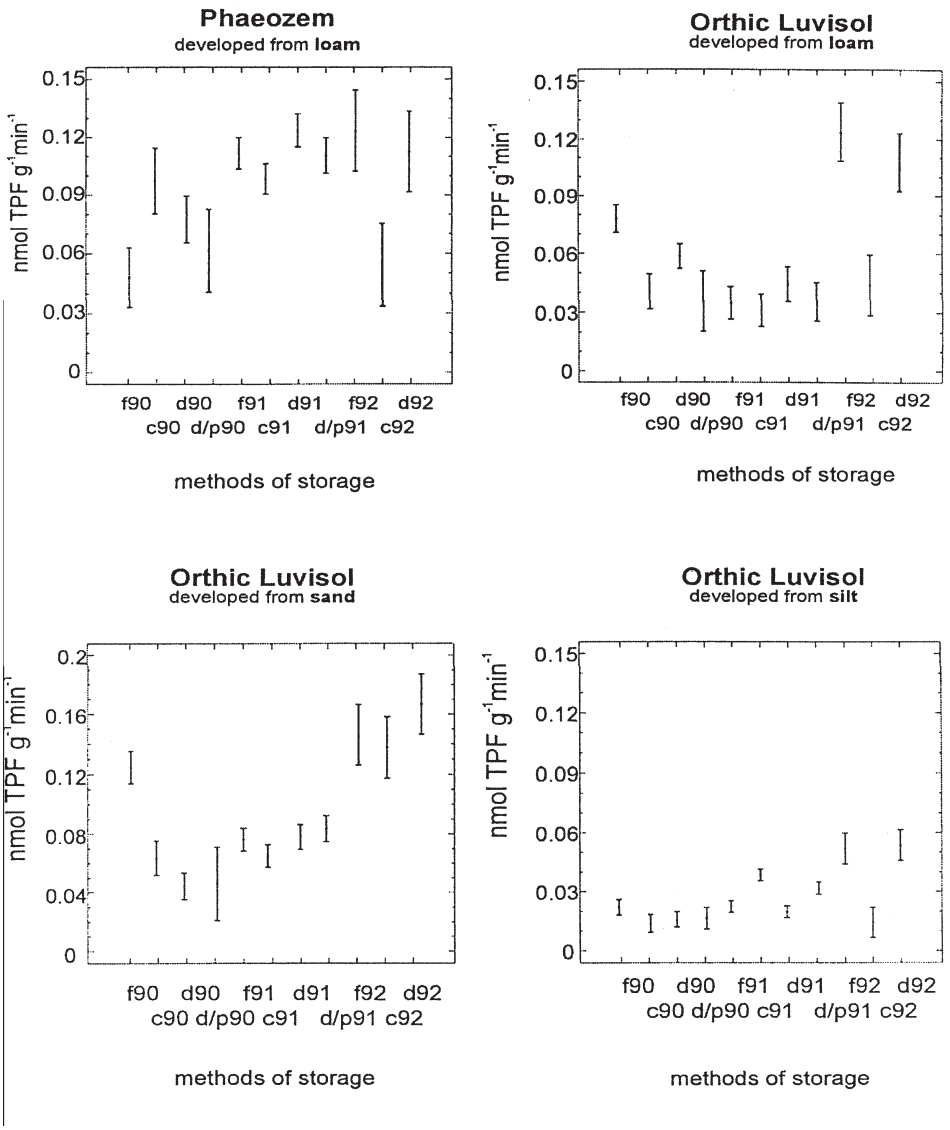

Fig. 9. Average values of dehydrogenase activity of the four types of soils sampled from 1990 to1992 (jointly for all periods of storage) as a function of the method of storage (f - fresh soil, c - cooled, d - air-dry and d/p - air-dry preincubated, 90, 91, 92 - year of sampling). The bars represent 95% LSD method.

Figure 10 shows seasonal differentiation of dehydrogenase activity of the Orthic Luvisol developed from silt sampled from 1991 to 1992 and - for comparison - the same soil samples stored under air-dry conditions for about 7 years. The activity was higher but the seasonal differentiation remained. It seems that dehydrogenase activity is affected by the environmental properties in the field so strongly that it "keeps" its initial activity for a long time. Differentiation between the activity of soil samples taken in different seasons of the year is maintained during the entire storage period. Figure 11 shows that there is a significant correlation between dehydrogenase activity of the fresh samples of the Orthic Luvisol developed from silt taken from October 1991 to September 1992 and those stored under air-dry conditions for seven years. Similar results were obtained in the experiment with the other soil types (Table 3).

According to Ross [15] it is desirable to determine, whether changes in the level of dehydrogenase activities that occur during soil storage are important in relation to other factors affecting the levels of these activities in the field. Skujinš [21] was of the opinion that general rules could not be formulated for the most suitable storage procedures to retain enzyme activities in the soil because of the differences between various enzymes or soils. Skujinš and McLaren [23] found that dehydrogenase activity was still detectable in some air-dried soils that had been stored for a few years, but rates or directions of changes in the activity related to storage were not recorded.

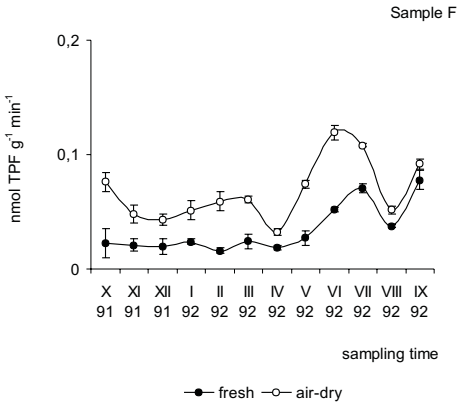

Fig. 10. Influence of drying and storage period on the dehydrogenase activity of the Orthic Luvisol developed from silt sampled from October 1991 to September 1992 (after about 7 years of storage in the air-dry conditions).

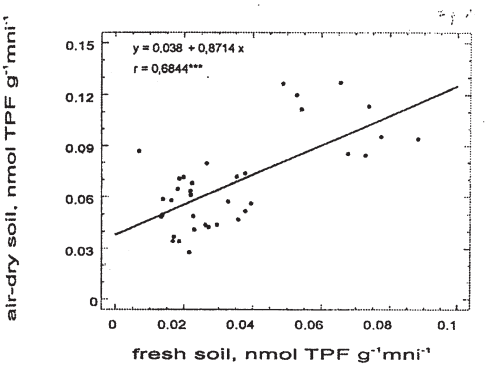

Fig. 11. Dehydrogenase activity of the Orthic Luvisol developed from silt stored under air-dry conditions for about 7 years in relation to the fresh soil sampled from October 1991 to September 1992. (Significance level of 0.001).

CONCLUSIONS

1. Dehydrogenase activity after preincubation of air-dried samples showed that there is no strong evidence of the formation of some characteristic activity of the soil enzyme related to with the type of soil, irrespective of the time and kind of storage and season of sampling for the Phaeozem developed from silty light loam and heavy loam and the Orthic Luvisol developed from silty light loam, sand and silt.

2. Differentiation among dehydrogenase activities of the soil samples under investigated conditions is maintained during the entire storage period lasting for up to about two years and depended on the type of soils and time of storage and sampling.

3. The present investigations demonstrate that the main factor affecting the levels of dehydrogenase activity is the sampling time.

Differentiation between the activity of the Orthic Luvisol, developed from silt taken in different season of the year, is maintained during the entire storage period (about 7 years).

4. Analysis of the seasonal patterns for the Orthic Luvisol developed from silt showed the highest dehydrogenase activity in September and the lowest in winter.

5. The highest dehydrogenase activity of fresh soils was found in Phaeozem developed from silty light loam and heavy loams. It was about four times higher than in the Orthic Luvisol developed from silt. This tendency was maintained during the whole experiment irrespective of the time and way of storage.

6. Air-drying resulted in a considerable decrease of dehydrogenase activity after storage for a period from one to two months with a tendency to increase with the storage time exceeding about 20 months.

7. The soil samples air-dried at room temperature and than preincubated showed more a stable dehydrogenase activity than other methods of storage with a slight tendency to increase in comparison to the air-dried and fresh soil samples.

8. Dehydrogenase activity of the undried samples kept at 4 ^{o}C (cooled) for about one month was similar to that activity in the fresh samples with a significant decline after this period of storage. Our results suggest that the worst method of storage for the investigated soils is storage at 4 ^{o}C for a period longer than 1 month.

9. Further studies should relate the results obtained to the biological activity of the soil in individual agrobiological soil units essential for food production.

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