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RESULTS OF MICROBIOLOGICAL ANALYSIS RELATED TO SOIL PHYSICAL PROPERTIES

M. Dąbek-Szreniawska

Institute of Agrophysics, Polish Academy of Sciences,
Doświadczalna 4, 20-236 Lublin, P.O. Box 121, Poland

A b s t r a c t. The numbers of microorganisms in separate soil size fractions (1-0.2, 0.2-0.02, 0.02-0.005 mm) were determined. The numbers of bacteria per 1 g or 1 cm^3 increased with a decrease in particle size: the increase being, however, lower than the corresponding increase in the surface area of the particle. The differences in the number of bacteria for the same fractions are very likely the result of different organic matter contents. Consequently, the highest number of bacteria were found in the humus-rich, medium loam. The amount of bacteria depends on the culture medium used in experiment: the amount of bacteria on the DNB medium was higher than that on the soil extract medium. The amount of cellulolytic bacteria is about 10-fold lower in sandy loam when compared to the soils of a heavier texture. CO_2 production following the amendment of soil with cellulose was not correlated with the number of cellulolytic bacteria developing under the conditions of the culture. Sacharase activity depend on the soil texture: the lowest values were found in sandy loam, the highest in medium loam.

INTRODUCTION

Investigations of soil microorganisms were based mainly on microbe-number, their distribution in the soil profile, and their biological activity.

There is, however, an interaction between microbial cells, colonies, and soil particles. One of these interactions is mechanical entrapment of microorganisms in narrow soil pores during filtration through the soil profile. Another form of the interaction is the concentration of microbial cells around the soil particles. According to Zvyagintsev [16] microbial interaction with soil particles makes the following processes possible:

(i) Adsorption of microbial cells on the surface of soil particles which is the most common phenomenon.
(ii) Aggregation of these components causing the formation of conglomerates.
(iii) Adsorption of fine soil particles on the surface of microbial cells.

According to Marshall [10] most of the soil microorganisms exist in the adsorbed state. The ability of various soils to adsorb bacteria differs in relation to their texture, and organic matter content and is related to the soil surface area and the kinds of electrical charges on the clay particles [4,5,10,17].

Sorption of microorganisms in soil is an important factor of biological life. There is a need to expand these studies in order to explain many of the important features of microbial life in soils.

The aim of the paper was to determine the number of adsorbed bacteria on soil particles of different sizes. Moreover, an attempt was made to assess the influence of sorption on biological activity of adsorbed cells.

MATERIALS AND METHODS

Soils

Three soils were used in the studies: a silty loam, a medium loam and a sandy loam. The soil characteristics are presented in Table 1.

Table 1. Soil characteristics

Soil	Particle size (mm) distribution (%, w/w)				Bulk density	Organic matter content	pH in
	1.0 - 0.1	0.1 - 0.02	0.02 - 0.002	< 0.002	($Mg\ m^{-3}$)	(%, w/w)	H_2O
Sandy loam	27	40	26	6	1.39	1.37	4.7
Medium loam	41	12	17	30	1.15	1.70	7.6
Silty loam	57	24	14	5	1.47	1.10	5.0

Media

Bacterial counts were made in the following media:

(a) A medium containing a soil extract: 100 ml soil extract [14], 1 g glucose, 0.5 g K_2HPO_4, 900 ml dist. water, 10 g agar; pH 7.

(b) DNB [6]: 0.1 g pepton, 0.1 g nutrient broth Difco, 0.05 g NaCl, 1 000 ml dist. water, 10 g agar.

(c) Tubes containing filter paper as the sole carbon source and a 5 ml solution containing 1 g $(NH_4)_2SO_4$, 1 g K_2HPO_4, 0.5 g $MgSO_4$, 2 g $CaCO_3$, NaCl traces, 1 000 ml distilled water.

The most probable number of bacteria was estimated according to the method of Rodina [14].

Determination of soil enzymatic activity

Fresh soils were sieved through a 1 mm sieve and used for the experiments.

Cellulase and sacharase activity was estimated according to the method of Russel [15].

Microbiological analyses

Twenty five milliliters of sterilized water was added to 1 g of fresh sieved soil. This suspension was shaken for 2 h in a laboratory shaker. Then, the suspension was adjusted to a volume of 100 ml. Ten milliliters were taken from a depth of 10 cm at time intervals corresponding to the settling of particle sizes 1-0.2 mm, 0.2-0.02 mm, and 0.02-0.005 mm [3]. These fractions with adsorbed bacterial cells were diluted and cultured on media.

The number of bacteria in 1 cm^3 was calculated by multiplying the number of bacteria in 1 g of the soil dry weight by soil bulk density [9]. The number of bacteria per cm^2 of soil surface area was calculated by dividing the number of bacteria in 1 g of soil by soil specific surface area expressed in $cm^2\ g^{-1}$ [9].

The theoretical specific area of a particular soil fraction was calculated assuming a spherical shape of the particles and using the mean diameter of these particles.

Statistical computations were made according to Oktaba [12].

RESULTS AND DISCUSSION

The number of bacteria attached to particles of three size fractions are presented in Table 2, while statistical computations are presented in Tables 3 and 4.

All the other experimental data are presented in Tables 5-9 and Figs 1-2.

Table 2. Number of bacteria in 1 g dwt of three size fractions cultured on medium with soil extract and on DNB medium (x 10^8)

Medium	Soil	Size of soil fractions (mm)			
		1 - 0.2	0.2 - 0.02	0.2 - 0.005	average
Soil extract	Sandy loam	0.37	1.04	5.50	2.30
	Medium loam	6.05	10.33	14.12	10.17
	Silty loam	0.41	0.52	4.67	1.53
DNB	Sandy loam	0.72	2.17	8.6	3.83
	Medium loam	5.63	12.44	9.5	9.19
	Silty loam	1.65	2.82	15.6	6.69

Table 3. Test function values obtained in analyses of variance for bacterial counts on soil extract and on DNB medium

Medium	Variability source	Degree of freedom	Test function	Boundary function $F_{0.05}$	Significance
Soil extract	Fractions	2	13.73	3.32	+
	Soils	2	478.80	3.32	+
	Error	31			
DNB	Fractions	2	1.80	3.32	-
	Soils	2	94.22	3.32	+
	Error	31			

+ significant; - not significant.

Table 4. Significant differences between the number of adsorbed bacteria cultured on medium with soil extract and on DNB medium

Compared soils	On soil extract	On DNB
Medium loam x silty loam	+	+
Medium loam x sandy loam	+	+
Silty loam x sandy loam	-	+

+ significant differences in favour of first soil; - not significant.

Table 5. Number of bacteria expressed per 1 mm^2 of the soil surface cultured on medium with soil extract and on DNB medium (x 10^7)

Medium	Soil	Size of soil fractions (mm)		
		1 - 0.2	0.2 - 0.02	0.02 - 0.005
Soil extract	Sandy loam	0.067	0.030	0.022
	Medium loam	0.920	0.250	0.047
	Silty loam	0.079	0.016	0.020
DNB	Sandy loam	0.133	0.064	0.035
	Medium loam	0.850	0.300	0.032
	Silty loam	0.320	0.087	0.067

Table 6. Number of sorbed bacteria expressed per 1 cm^3 of the soil fractions cultured on soil extract and on DNB medium (x 10^7)

Medium	Soil	Size of soil fractions (mm)		
		1 - 0.2	0.2 - 0.02	0.02 - 0.005
Soil extract	Sandy loam	5.09	14.43	76.49
	Medium loam	69.60	118.87	162.48
	Silty loam	6.01	7.60	68.63
DNB	Sandy loam	10.05	30.18	119.61
	Medium loam	64.70	143.15	109.29
	Silty loam	24.29	41.52	229.41

Table 7. Distribution of the number of bacterial cells (per 1 g of dry matter) in different soil size fractions (x 10^8)

Medium	Soil	Size of soil fractions (mm)			In 1 g d.m.
		0.1-0.2	0.2-0.02	0.02-0.005	
Soil extract	Sandy loam	0.2086	0.2491	0.7700	1.2277
	Medium loam	2.4805	1.2396	3.6712	7.3913
	Silty loam	0.1104	0.2068	1.2113	1.5306
DNB	Sandy loam	0.4120	0.5210	1.2040	2.1370
	Medium loam	2.3080	1.4930	1.6150	5.4160
	Silty loam	0.4455	1.1280	4.0560	5.6296

The number of microorganisms expressed on a soil mass or volume basis increased 1.6-15 times but that expressed on a particle surface area basis decreased 3-38 times with decreasing particle size (Table 8).

The problem of determining the number of microorganisms adsorbed in soil is a basic one which has been disscused by many authors. The number of countable microorganisms is dependent on the experimental

Table 8. Ratio of the bacterial counts in the finest soil fraction (0.02-0.005 mm) to that in the coarsest one (1-0.2 mm) as expressed on a soil weight or a soil volume basis and on a soil surface area basis

Soil	Ratio of microbal counts expressed			
	per 1 g or 1 cm^3		per 1 cm^2	
	DNB	Soil extract	DNB	Soil extract
Sandy loam	12.0	15.0	$\frac{1}{38}$	$\frac{1}{3}$
Medium loam	1.6	2.4	$\frac{1}{27}$	$\frac{1}{20}$
Silty loam	9.5	11.4	$\frac{1}{5}$	$\frac{1}{4.7}$

Sacharase activity presented in Fig. 1, and cellulolytic activity in Fig. 2 was not correlated with the number of cellulolytic bacteria.

conditions: the kind of soil, the kind of nutrient medium, the medium used for the soil suspension and finally, the type of agar used [2,6,7,8,11]. For these experiments the kind

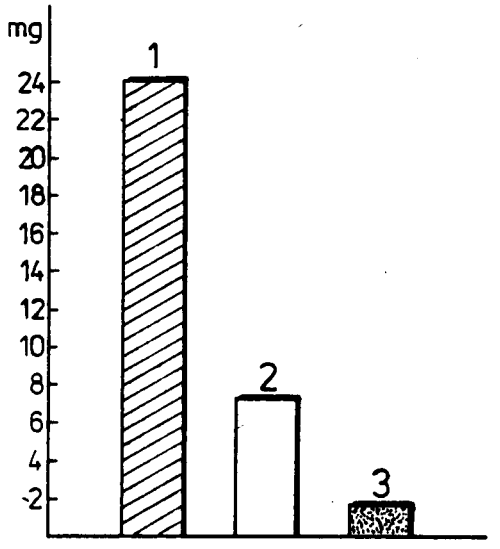

Fig. 1. Sacharase activity in mg of decomposed substrate (saccharose) per 1 g of soil (dwt): 1 - medium loam, 2 - silty loam, 3 - sandy loam.

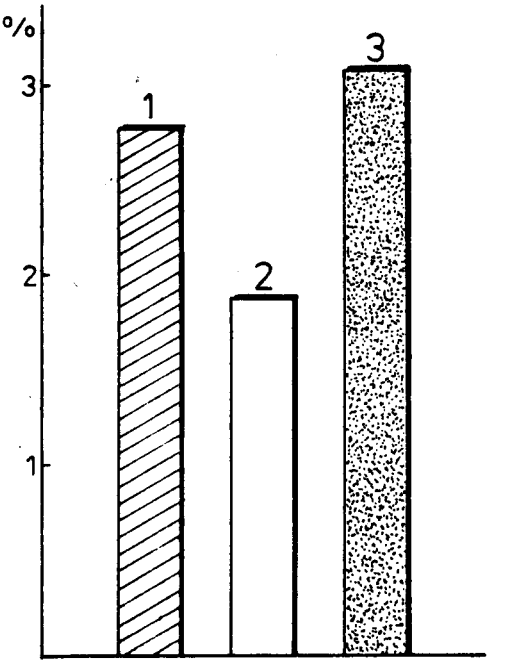

Fig. 2. Cellulolytic activity in % of added cellulose carbon evolved as CO_2 per 1 g of soil (dwt): 1 - medium loam, 2 - silty loam, 3 - sandy loam.

of soil has an essential influence on the number of adsorbed bacteria. There is a significant difference in the number of bacteria adsorbed on different soils: the highest bacterial counts occur on the medium loam (Tables 2, 5-8). Burrichter [1] found the highest microbial counts on particle sizes of 0.2-0.025 mm and little on coarse particles in a humus-rich forest soil. Marshall [10] stated that heavy textured soils adsorb more bacteria. The presence of clay particles or organic substance allows for a greater number of bacteria on these particles. Zvyagintsev [16] in studies on a humus-gley soil pointed out that the number of microbes on soil particles was directly related to the particle size within the range 0.001-0.5 mm while a soddy soil showed the lowest number of microbes on coarse particles (0.4-0.5 mm) when compared to those estimated theoretically. For these experiments there is also a relation between the size of the soil particles and the microorganisms. The number of bacteria differed both between the size fraction and for the same fractions derived from different soils: the smaller size fraction the greater number of bacteria.

Table 9. Number of cellulolytic bacteria in different soil size fractions per 1 g of dry weight ($\times 10^7$)

Soil	Size of soil fractions (mm)			
	1 - 0.2	0.2 - 0.02	0.02 - 0.005	Mean
Sandy loam	0.23	0.71	0.27	0.41
Medium loam	5.26	5.28	1.87	4.14
Silty loam	2.61	4.17	5.00	1.70

In all cases, the highest number of bacteria were found in the clay fraction (0.02-0.005 mm) (Tables 2, 5-8).

The highest number of bacteria were found in the humus-rich medium loam. An exception to this is the high value (4.056×10^8) of the number of bacterial cells on DNB for the 0.02-0.005 mm fraction of the silty loam (Table 7).

According to Hattori [4] the majority of particles of 0.02-0.01 mm diameter adsorbed 1-100 bacterial cells in fresh soil. In the case of soil particles smaller than 0.01 mm, the number of bacteria occurring on their surface was proportional to the square of a diameter of the particle [5].

There is a relationship between bacteria number and kind of medium used. The DNB medium allowed for a greater number of bacteria than the medium from the soil extract (Tables 2, 5-8). The number of bacteria in the case of DNB was larger for almost all soil fractions when compared to the soil extract medium: in sandy loam soil the factors are 1.97, 2.09, and 1.56 for fractions 1-0.2, 0.2-0.02, and 0.02-0.002, respectively; average 1.87; in the medium loam soil: 0.93, 1.20, and 0.67 with an average 0.93; and in the silty loam 4.03, 5.46, and 3.34; average 4.27. These results correspond with those obtained by Hattori [6] and Ohta and Hattori [11] who noticed that a nutrient broth (NB) medium gave lower colony counts than its 100-fold dilution (DNB). Media containing low concentrations of organic substances gave higher colony counts than those containing high concentrations of organic substances.

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WYNIKI BADAŃ MIKROBIOLOGICZNYCH W ODNIESIENIU DO WŁAŚCIWOŚCI FIZYCZNYCH GLEB

W trzech gatunkach gleb: piasek gliniasty, glina średnia i glina pylasta zaobserwowano wzrost liczebności bakterii wraz ze zmniejszeniem się wielkości frakcji granulometrycznych. Zróżnicowana liczebność bakterii prawdopodobnie zależna była od zawartości substancji organicznej. Najwyższą liczebność bakterii stwierdzono w glinie średniej, bogatej w substancje próchniczne. Stwierdzono także zróżnicowanie liczebności bakterii w zależności od zastosowanego podłoża hodowlanego. Najwyższe liczebności bakterii stwierdzono na podłożu DNB.