Int. Agrophysics, 2006, 20, 277-288

INTERNATIONAL
Agrophysics
www.ipan.lublin.pl/int-agrophysics

Numbers of culturable bacteria in soil under mineral or organic cultivation: comparison of Hattori's 'FOR' and standard dilution plate methods**

M. Dąbek-Szreniawska[1], M. Hajnos[1], G. Stotzky[2], Y. Collins[2], and J. Malicki[3]*

[1]Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, P.O. Box 201, 20-290 Lublin 27, Poland
[2]New York University, Biology Department, Laboratory of Microbial Ecology, 100 Washington Square East, New York, NY 10003-6688, U.S.A.
[3]Technical University, Nadbystrzycka 40, 20-618 Lublin, Poland

Received June 28, 2006; accepted September 5, 2006

A b s t r a c t. Two concepts and methods for determining the number of culturable bacteria were compared in soil under conventional *ie* mineral fertilizers, herbicides, fungicides or organic management *ie* manure or compost, mechanical or manual weeding. In the first method, colony-forming units (CFUs) of bacteria were counted after 14 days of incubation of soil dilutions on two different media *ie* diluted nutrient broth agar (DNBA) and soil extract agar (SEA). In the second method, the First Order Reaction (FOR) model was used to determine the CFUs of bacteria that were counted 7 times on successive days after plating the soil dilutions on the media. The CFUs were also expressed on the basis of 1 g of fresh soil, 1 g of oven-dry soil, 1 cm^3 of soil, 1 cm^3 (ml) of soil dilution, 1 cm^3 of soil porosity, and 1 cm^2 of pore surface. The numbers of bacteria were compared with the organic carbon content of the soils, as soil organic matter was assumed to be the major substrate for the growth of the bacteria. The content of carbon was 0.96% (in soil receiving mineral fertilizers) and 1.34% (in soil receiving organic fertilizers), resulting in a comparative ratio of 0.96:1.34 = 0.72.

K e y w o r d s: soil bacteria, methods of determining bacterial numbers, first order reaction (FOR) model.

INTRODUCTION

The numbers of bacteria in soil have been determined by both direct and indirect methods. Direct methods *eg* microscopic observations generally do not provide adequate information about the physiologic and taxonomic characteristics of the bacteria. Indirect methods *eg* plating, immunological, molecular, measurements of microbial activity also have deficiencies. Methods that determine the number of physiological groups by measuring the metabolites produced *eg* CO_2, NH_3, fatty acids from the introduction of specific substrates can be biased as a result of errors in interpreting the kinetics of the production of metabolites. Molecular and immunological methods can provide considerable information on community structure and microbial diversity, but many of the organisms detected may be nonculturable, thus preventing their precise identification. The number of bacteria determined by serial dilution and plating on different media depends on:

– how soil samples are stored before the preparation of the dilutions (Stotzky *et al*., 1962);
– the suitability for growth of the media (Hattori and Hattori, 1980); and
– the interpretation of the results (Malicki, 1980; 1981; Dąbek-Szreniawska, 1992).

The purpose of this study was to compare two concepts and methods of determining the number of culturable bacteria in a soil under different methods of fertilizing: one receiving mineral fertilizers and the other receiving organic fertilizers. In the first method *eg* Parkinson *et al*., 1971, the colony-forming units (CFUs) of bacteria were counted after a specific period of incubation of soil dilutions (14 days) on different media. In the second method, the First Order Reaction (FOR) model (Ishikuri *et al*., 1984; Ishikuri and Hattori, 1987; Hattori, 1988; Hattori and Hattori, 2000) was used to determine the CFUs of bacteria which were counted 7 times on successive days for 14 days after plating the soil

*Corresponding author's e-mail: mdsz@ipan.lublin.pl
**This work was partly financed by budget support for science in Poland, Grant No. 5PO6B 007 10 and was done primarily in the Laboratory of Microbial Ecology, New York University.

dilutions on different media. Although counting the numbers of CFUs only once after a definite period of incubation saves time, this method provides only limited information. In contrast, the FOR model, although requiring more time, provides information on the kinetics of colony growth, on the suitability of the medium for the growth of the bacteria, on the time of appearance of the first colony, and on the potential number of CFUs in the soils.

The FOR model provides an equation that describes the relation between the number of colonies and incubation time:

$$N(t) = N\infty\,[1-\exp(-\lambda(t-t_r)], \quad t \geq t_r,$$

where: $N(t)$ and $N\infty$ are numbers of colonies observed at time t and after an infinite time; t_r (retardation time) is a parameter reflecting the delay or lag in growth; and λ is a parameter indicating the rate of colony formation. This model allows one to estimate the number of colonies that are potentially able to grow on the medium used. The time required for the appearance of the first colony and the rate of the increase of the number of colonies are also characteristics of the FOR model.

Hattori (1988) and Hattori and Hattori (2000) described how to estimate the parameters mentioned above. According to the FOR model, it is possible to estimate $N\infty$ from counts made on successive days. In the FOR model, the minimum number of counts of CFUs is three on three successive days. Hattori and Hattori (2000) also described the possibility of estimating the value of $N\infty$ from counting the data in a much shorter period than that of the standard method. Moreover, they discussed how to estimate $N\infty$ for a mixed population of bacteria in a complex environment such as soil. Based on the FOR theory, the parameter t_r may be estimated because there is a linear relation between the division rate and t_r, suggesting that t_r is closely, but not exactly, related to the division rate of an organism. Moreover, the FOR theory indicates that, generally, the t_r value can be divided into two parts: the first part is the time before cell division begins, and the second part is the time required for a dividing cell to produce a colony of a visible size which is affected by several factors, such as the generation time of the organism, the spatial arrangement of 'newly born cells on the solid medium', the production of slime substances by the cells, and the 'threshold size for the visual detection of the colony'. Hattori and Hattori (2000) also discussed how to estimate the parameter λ which corresponds to the rate constant in first-order kinetics and, in terms of statistical analysis, is defined as 'the probability of forming colonies in an interval of unit time'. The parameter λ reflects the rate, or the probability, for the initiation of cell division in a unit of time. Moreover, they reported that λ depends greatly on the age of the inoculum and is the highest when inoculated cells are in the 'exponential phase' and becomes lower when cells are in the stationary and death phases.

We evaluated the FOR model to see how the serial dilution technique in the enumeration of culturable soil bacteria from the soil treated with conventional *ie* mineral fertilizers, herbicides, fungicides or organic management *ie* manure or compost, mechanical or manual weeding is affected by the composition of the media, by the method of expressing the number of CFUs, and the interpretation the results.

MATERIALS AND METHODS

Soil samples from an Orthic Luvisol (FAO classification) were obtained from fields planted long-term with wheat at the Institute of Soil Science and Plant Cultivation in Puławy, Poland (Kuś, 1998). The field experiments utilized two crop management as systems: conventional *ie* mineral fertilizers, herbicides, and fungicides and organic *ie* fertilized with manure or compost and with mechanical and manual weeding. Some physicochemical characteristics of the soil samples are presented in Table 1. The content of organic carbon was determined by the methods of Tiurin (1931); total porosity, total pore volume, and bulk density with a Carlo Erba Mercury Porosimeter Series 2000 as described by Hajnos *et al*. (1998); specific surface area by water-vapour adsorption (Sokołowska *et al*., 1998); pH and water content by the methods of McLean (1982). Other physicochemical characteristics of the soils have been described by Sokołowska *et al*. (1999) and Dąbek-Szreniawska *et al*. (2002).

T a b l e 1. Physicochemical characteristics of the soils

Soil property	Mineral fertilizers	Organic fertilizers	Ratio (M : O)
Organic carbon content (%)	0.96	1.34	0.72
Bulk density (g cm^{-3})	2.3	2.1	1.1
pH (in H_2O)	6.7	7.5	–
Total pore volume (cm^3 g^{-1})	40.9	68.1	0.60
Total porosity (%)	9.4	14.3	0.66
Water content (%)	53.0	60.0	0.88
Specific pore surface area (m^2 g^{-1})	0.43	0.83	0.52

Mercury intrusion porosimetry (MIP)

This technique involves forcing of mercury into brown coals samples at increasing hydraulic pressure. Because mercury does not adhere to most of solids (the contact angle is higher than 90°), it enters the pores only when an external pressure (p_m) is applied (for example water of contact angle close to 0° enters the pores spontaneously). The higher the pressure, the narrower the pores mercury is forced into. The pores should be empty at the beginning, and so the sample is out-gassed at a vacuum prior to the mercury intrusion. The MIP apparatus registers the volume of mercury forced into the sample against the intrusion pressure $V = V(p_m)$. This volume V is related directly to the pore volume, and the intrusion pressure may be related to the (equivalent) pore radius using the Washburn equation:

$$r_0 = -2\gamma_m \cos\theta_m / p_m,$$

where: γ_m – is mercury surface tension, θ_m – is mercury-solid contact angle (for soils this equals to 141.3°).

The volume of pores having radius less than given r_0 is calculated as:

$$V(r < r_0) = V_0 - V_s - V(p_m),$$

where: V_s – is volume of the solid phase of the porous body, V_0 – is volume of all pores before the intrusion of mercury and $V(p_m)$ is volume of the intruded mercury at a pressure p_m.

The pore size and pore volume distributions were determined in the range from 3.7 to 7500 nm radius by MIP as described by Hajnos and Świeboda (2004). Cumulative pore size distributions and pore size distributions were analysed. The measurements were done using the Carlo Erba 2000 porosimeter. The surface tension and the contact angle of mercury were assumed to be 480 mJ m^{-2} and 141.3, respectively. Using the computer programme Milestone 100, and assuming the cylindrical pore model, such parameters as: bulk density, pore specific surface area (PSSA), and total porosity (TP) were calculated.

Structural analyses of soil samples were done in five replications. Before porosimetric analyses, the samples were oven-dried at 105°C and then outgassed up to 10^{-3} Pa to remove physically adsorbed water from their surface.

Between microbiological experiments, the soil samples were kept at 4±1°C. Serial 10-fold dilutions of the soils were made daily for 6 days and inoculated into plates with the medium that were incubated at 25 ± 1°C for 14 days. Colonies were counted at 1, 2, 3, 5, 7, 10, and 14 days after inoculation (Tables 2 and 7a,b,c,d), and the number of CFUs of bacteria was determined on the basis of the FOR model. The number of CFUs of bacteria was also determined once after 14 days of incubation (Parkinson *et al*., 1971). The following media were used: Diluted Nutrient Broth + agar (DNBA) (Hattori and Hattori, 1980) containing (per litre): 0.1 g peptone, 0.1 g beef extract, 0.05 g NaCl, and 15 g agar; and Soil Extract Agar (SEA) containing 100 ml of soil extract and 0.2 g K_2HPO_4, 1 g dextrose, 15 g agar, and 900 ml tap water (Stotzky *et al*., 1993).

The CFUs were expressed on the basis of 1 g of fresh soil, 1 g of oven-dry soil, 1 cm^3 of soil, 1 cm^3 (ml) of soil dilution, 1 cm^3 of soil porosity, and 1 cm^2 of pore surface.

Examples of calculations: SEA, Organic fertilizer, Standard (Table 5): N CFUx10^6 g^{-1} fresh soil = 159.7 *eg* SEA and organic fertilizers:
N CFU x10^6 g^{-1} dry soil = (100% / 100% - % moisture) (N CFU x10^6 g^{-1} fresh soil);
(100% / 100% - 60%) 159.7 = (100% / 40%) 159.7 = 399.2
N CFU x10^6 cm^{-3} soil = bulk density (N CFU x10^6 g^{-1} dry soil); 2.1x 399.2 = 838.3
N CFU x10^6 cm^{-3} soil solution = (100% / moisture %) (N CFU x10^6 g^{-1} fresh soil);
(100% / 60%) 159.7 = 266.1
N CFU x10^6 cm^{-3} porosity = (1000 mm^{-3} / porosity) (N CFU x10^6/ g dry soil);
(1000 / 68.1) 399.2 = 5861.2
(N CFU x10^6 cm^{-2} surface of pores = (1 / surface of pores) N CFU x10^6 g^{-1} dry soil;
(1 / 8300 cm^2) 399.2 = 0.048.

The CFUs of bacteria were related to the organic carbon content of the soils, as soil organic matter was assumed to be the major substrate for the growth of the bacteria (Monod, 1950). Statistical analyses of CFUs were done according to Snedecor (1956), Dmitriev (1972), and Parkin and Robinson (1994). The agreement between the results of analysis of replicates of the same soil samples was determined by calculating Pearson's coefficient of variance (% CV) for each individual series which determines the percentage of the means that constitutes the standard deviation. The equality of variance was determined by the Fisher-Snedecor test (Snedecor, 1956).

RESULTS AND DISCUSSION

The soils differed in their organic carbon depending on the type of fertilization (Table 1), which apparently influenced the numbers of bacteria.

The results obtained with the FOR model are presented in Tables 2 and 4. According to the FOR model, the bacteria were counted on a minimum of three successive days after making dilutions and inoculating the media: the media were inoculated daily for six consecutive days. The highest number of CFUs was obtained from the organically-fertilized soil on DNBA, and the lowest number of CFUs was obtained on SEA from the minerally fertilized soil. Counts on the first and second days on SEA were relatively low, suggesting that SEA does not support well the growth of rapidly growing bacteria. The media used influenced the number of CFUs and also the time of the appearance of the first colony. The appearance of the first colony, t_r, was earlier on DNBA than

T a b l e 2. Colony forming units (CFUs) 10^6 g^{-1} of fresh soil according to the FOR model

Day of diluting and inoculating	Estimated parameters (FOR model) $N(t) = N\infty[1-\exp(-\lambda(t-t_r)], \quad t \geq t_r$			Standard error	Correlation coefficient
	$N\infty$	t_r	λ		
Soil with mineral fertilizers cultured on DNBA, moisture content of 53%					
1	176.4	0.307	0.21745	12.956	0.971
2	126.5	1.022	0.24348	7.363	0.987
3	141.6	1.032	0.21331	6.261	0.992
4	160.8	0.948	0.08159	5.867	0.988
5	166.8	0.719	0.23345	6.492	0.993
6	118.4	0.756	0.27334	9.567	0.973
Mean	148.41	0.797	0.21043		
SD	21.2217	0.2505	0.06087		
Soil with mineral fertilizers cultured on SEA, moisture content of 53%					
1	142.2	0.723	0.08634	6.665	0.980
2	99.6	0.938	0.17472	11.954	0.936
3	99.0	1.324	0.10533	10.851	0.933
4	116.5	1.333	0.08208	12.723	0.914
5	196.7	1.326	0.08935	10.751	0.978
6	102.3	0.871	0.12988	5.563	0.985
Mean	126.05	1.0858	0.11712		
SD	34.97	0.2500	0.003267		
Soil with organic fertilizers cultured on DNBA, moisture content of 60%					
1	420.0	0.131	0.17778	19.047	0.986
2	348.9	0.514	0.20645	16.119	0.989
3	327.0	0.603	0.21283	19.308	0.984
4	253.6	0.648	0.17079	12.023	0.989
5	330.7	0.587	0.20437	8.713	0.997
6	299.6	0.227	0.32743	22.466	0.968
Mean	329.96	0.4516	0.21661		
SD	50.378	0.19872	0.05189		
Soil with organic fertilizers cultured on SEA, moisture content of 60%					
1	234.6	1.106	0.13760	16.832	0.973
2	258.6	1.247	0.14706	19.788	0.972
3	282.7	1.518	0.08185	15.047	0.977
4	157.7	1.102	0.15617	9.404	0.987
5	198.4	1.102	0.13118	9.278	0.989
6	330.1	1.067	0.23315	10.772	0.996
Mean	243.683	1.1903	0.14783		
SD	55.881	0.15732	0.04488		

T a b l e 3. Colony forming units (CFU) 10^6 g^{-1} of fresh soil counted once after 14 days of incubation; standard method

Day of diluting and inoculating		Mineral fertilizers		Organic fertilizers	
		DNBA	SEA	DNBA	SEA
1	$\bar{x} \pm SD$	165.5±10.2	93.8±10.3	387.4±23.1	193.8±21.6
	n	5	5	5	4
	%*CV*	6.2	11.1	5.9	11.1
2	$\bar{x} \pm SD$	118.3±2.77	87.6±17.0	329.2±19.1	210.0±20.7
	n	4	5	5	5
	%*CV*	2.3	19.5	5.8	9.9
3	$\bar{x} \pm SD$	130.7±4.2	70.3±14.2	311.0±14.5	172.8±15.2
	n	3	3	3	4
	%*CV*	3.2	20.2	4.6	8.8
4	$\bar{x} \pm SD$	99.7±3.8	72.3±15.1	233.3±13.7	131.3±1.7
	n	3	3	3	3
	%*CV*	3.9	20.8	5.9	1.33
5	$\bar{x} \pm SD$	158.7±7.4	127.7±9.7	312.0±7.1	159.7±10.5
	n	3	3	3	3
	%*CV*	4.6	7.6	2.3	6.6
6	$\bar{x} \pm SD$	115.7±10.6	80.3±4.5	309.0±15.5	309.7±9.9
	n	3	3	3	3
	%*CV*	9.2	5.6	5.0	3.2

$\bar{x}$ – mean, *SD* – standard deviation, % CV – coefficient of variance, % CV ~ $\frac{SD}{\bar{x}}100$, *n* – number of repetitions of the sample.

T a b l e 4. Colony forming units (CFUs)10^6 g^{-1} of fresh soil according to the FOR model

Days after making dilutations and inoculating		Mineral fertilizers (at 53% moisture content)		Organic fertilizers (at 60% moisture content)	
		DNBA	SEA	DNBA	SEA
1	N ∞	176.4±12.956	142.2±6.665	410.1±19.047	234.6±16.832
	%*CV*	7.34	4.68	4.53	7.17
2	N ∞	126.4±7.363	99.6±11.954	348.9±16.119	258.6±19.788
	%*CV*	5.82	12.00	4.62	7.65
3	$\bar{x} \pm SD$	141.6±6.261	99.0±10.851	327.0±19.308	282.7±15.047
	%*CV*	4.42	10.96	5.90	5.32
4	N ∞	160.8±5.867	116.5±12.723	253.6±12.023	15.77±9.404
	%*CV*	3.65	10.92	4.74	5.96
5	N ∞	166.8±6.432	196.7±10.751	330.7±8.713	98.4±9.278
	%*CV*	3.89	5.46	2.63	4.67
6	N ∞	118.4±9.567	102.3±5.563	299.6±22.466	330.1±10.772
	%*CV*	8.08	5.44	7.49	3.26

Explanations as in Table 3.

T a b l e 5. Colony forming units (CFU) 10^6 g^{-1} of soil by standard method and FOR model when keeping the soil samples for 5 days before inoculation of the dilutions

Number of CFUs of bacteria (N)	Mineral fertilizer				Organic fertilizer			
	DNBA		SEA		DNBA		SEA	
	Standard	FOR	Standard	FOR	Standard	FOR	Standard	FOR
N g^{-1} fresh soil	158.7	166.7	127.7	196.7	312.0	330.7	159.7	198.4
N g^{-1} dry soil	337.6	354.9	271.6	418.5	780.0	826.8	399.2	496.1
N cm^{-3} soil	776.4	816.4	624.7	962.6	1638.0	1736.0	838.3	1041.8
N cm^{-3} soil dilution	299.4	314.8	240.9	371.1	520.0	551.2	266.1	330.7
N cm^{-3} porosity	8259.7	8684.6	664.6	10240.1	11454.5	12142.2	5861.2	7285.4
N cm^{-3} pores surface	0.078	0.082	0.063	0.097	0.094	0.099	0.048	0.059

on SEA, and the growth kinetics, l, were faster on DNBA (Table 2). Although colonies appeared earlier from the organically-fertilized soil, the subsequent rate of development of colonies was essentially the same for both the methods of fertilization and calculation. Table 3 shows the number of CFUs that grew on DNBA and SEA and were counted only once after 14 days of incubation according to the standard method (Parkinson *et al*., 1971). Table 4 is based on the results presented in Table 2. The physicochemical data in Table 1 were used for the calculations in Table 5, which shows the numbers of CFUs of soil bacteria (taken from Tables 3 and 4 when they were made 5 days after collection of the soil samples the soil dilutions and inoculation). The 5th day was chosen for the calculations because it had the lowest value of %CV of Pearson's coefficient. In Table 5, the data in the first line (fresh soil) are taken from Tables 3 and 4. The second line is the CFUs g^{-1} of oven dry soil and these calculations are shown in the footnote. When the results were expressed on a basis other than 1 g of fresh soil (Table 5, using the data from Tables 1, 3, and 4), the numbers of CFUs differed not only in the methods of fertilization and cultivation and the method of measurement but also with how the results were expressed. The highest numbers of bacteria in soil were obtained when the results were expressed as the number of bacteria cm^{-3} of soil porosity, using the FOR model and the DNBA medium.

The importance of soil organic matter in environmental protection and for the nutrient supply of the plants has strongly decreased in the last decades (Korschens, 2004). In our experiments the ratio of soil bacteria and organic carbon content is discussed. Table 6 shows the comparison of the ratios of CFUs of bacteria and organic carbon contents from soil receiving mineral fertilizers to organically-fertilized soil (Table 5). The ratio of CFUs of bacteria in soil receiving mineral fertilizers to organically-fertilized soil that most closely approximated the ratio of the organic carbon content of minerally to organically-fertilized soil is also shown. The numbers of bacteria were compared with the organic carbon

T a b l e 6. Comparison of the ratio of CFUs of bacteria (from Table 5) and the ratio of organic carbon content of minerally and organically-fertilized soil

Number of CFUs of bacteria	Standard method		FOR model	
	DNBA	SEA	DNBA	SEA
	The ratio of organic carbon content of minerally fertilized soil to organic carbon content of organically-fertilized soil was 0.96:1.34 = 0.72			
N g^{-1} fresh soil	0.51	0.80	0.50	1.0
N g^{-1} dry soil	0.43	0.68	0.43	0.84
N cm^{-3} soil	0.47	0.74*	0.47	0.92
N cm^{-3} soil dilution	0.57	0.90	0.57	1.12
N cm^{-3} porosity	0.72*	1.13	0.71*	1.40
N cm^{-3} pores surface	0.83	1.31	0.83	1.64

*Ratio of CFUs of bacteria that most closely approximates to the ratio of organic carbon content of minerally- to organically-fertilized soil.

T a b l e 7a. Colony forming units (CFUs) 10^6 g^{-1} fresh soil with mineral fertilizers; cultured on DNBA

Day of diluting and inoculating	Sample number	Time of counting colonies (days)						
		1	2	3	5	7	10	14
1	1	28	37	67	119	138	156	169
	2	29	40	62	94	117	134	149
	3	31	49	84	125	144	158	167
	4	38	54	86	134	157	168	177
	Pearson's coefficient %CV	12.4	15.1	13.9	12.6	10.4	8.0	6.2
	Mean value of Pearson's coefficient: %CV =11.23 SD=2.97							
2	1	7	18	56	80	102	117	117
	2	5	12	56	74	107	118	123
	3	3	11	48[x]	71	92	111	116
	4	4	18	59	83	95	113	117
	Pearson's coefficient %CV	31.1	22.2	2.5	6.2	5.9	2.5	2.3
	Mean value of Pearson's coefficient: %CV=10.38 SD =10.66							
3	1	7	18	50	84	106	122	132
	2	5	12	50	80	99	117	125
	3	3	17	53[x]	81	110	125	135
	Pearson's coefficient %CV	32.6	16.7	0.0	2.1	4.3	2.7	3.2
	Mean value of Pearson's coefficient: %CV=8.8 SD =10.95							
4	1	6	9	26	42	74	89	105
	2	1	10	31	43	54	84	96
	3	3	10	25	36	53	83	98
	Pearson's coefficient %CV	61.6	4.9	9.6	7.66	16.0	3.1	3.9
	Mean value of Pearson's coefficient: %CV=15.25 SD =19.35							
5	1	11	48	75	106	135	161	168
	2	9	38	64	97	121	140	150
	3	9	44	77	103	128	148	158
	Pearson's coefficient %CV	9.7	9.5	7.9	3.7	4.5	5.8	4.6
	Mean value of Pearson's coefficient: %CV=6.53 SD =2.30							
6	1	9	36	46	68	85	95	103
	2	5	29	47	86	99	107	115
	3	8	44	60[x]	92	113	119	129
	Pearson's coefficient %CV	23.2	16.8	1.0	12.4	11.5	9.1	9.2
	Mean value of Pearson's coefficient: %CV=11.88 SD =6.39							
Mean value: %CV		28.43	14.20	5.81	7.45	8.76	5.20	4.90
Standard deviation		17.12	5.57	5.03	3.98	4.26	2.62	2.27

[x]Rejected with probability of 90% (Dmitriev, 1972).

T a b l e 7b. Colony forming units (CFUs) 10^6 g^{-1} fresh soil with mineral fertilizers; cultured on SEA

Day of diluting and inoculating	Sample number	Time of counting colonies (days)						
		1	2	3	5	7	10	14
1	1	11	16	26	42	68	88	94
	2	6	13	21	42	62	76	83
	3	7	11	21	39	58	76	82
	4	6	10	17	36	58	85	101
	5	8	15	24	43	65	93	109
	Pearson's coefficient %CV	24.4	17.5	14.0	6.4	6.3	8.0	11.1
	Mean value of Pearson's coefficient: %CV =12.53 SD=6.17							
2	1	3	10	23	44	54	64	69
	2	6	12	22	50	69	84	90
	3	9	14	32	53	70	84	92
	4	6	16	39	69[x]	89	107	116
	5	0	10	25	45	50	65	71
	Pearson's coefficient %CV	63.7	18.8	22.8	7.6	20.8	19.48	19.46
	Mean value of Pearson's coefficient: %CV=24.66 SD =16.57							
3	1	0	5	11	25	35	51	57
	2	1	7	13	45[x]	64	82	90
	3	0	4	9	26	38	57	64
	Pearson's coefficient %CV	1.4	23.4	14.8	1.9	28.5	21.2	20.2
	Mean value of Pearson's coefficient: %CV=35.91 SD =43.76							
4	1	3	4	14	31	62	75	83
	2	2	3	20	32	55	75	83
	3	1	1	2[x]	14[x]	27	40	51
	Pearson's coefficient %CV	40.8	46.8	46.9	1.6	31.1	26.0	20.8
	Mean value of Pearson's coefficient: %CV=30.57 SD =15.07							
5	1	1	4	13	50	69	106	118
	2	1	6	19	53	79	110	124
	3	7	14	17	62	93	130	141
	Pearson's coefficient %CV	94.3	54.0	15.3	9.3	12.25	9.1	7.6
	Mean value of Pearson's coefficient: %CV=28.83 SD =30.70							
6	1	3	6	26	42	62	75	83
	2	1	8	27	44	65	76	84
	3	0	6	21	40	58	64	74
	Pearson's coefficient %CV	93.5	14.1	10.6	3.9	4.6	7.6	5.6
	Mean value of Pearson's coefficient: %CV=19.98 SD =30.19							
Mean value: %CV		76.35	29.10	20.73	5.11	17.25	15.23	14.13
Standard deviation		38.67	15.44	12.25	2.87	10.29	7.28	6.25

[x]Explanations as in Table 7a.

T a b l e 7c. Colony forming units (CFUs) 10^6 g^{-1} fresh soil with organic fertilizers; cultured on DNBA

Day of diluting and inoculating	Sample number	Time of counting colonies (days)						
		1	2	3	5	7	10	14
1	1	76	134	170	234	284	314	356
	2	76	120	169	252	335	365	414
	3	84	104	166	245	318	362	413
	4	82	99	148	219	289	326	370
	5	89	104	160	250	300	331	384
	Pearson's coefficient %CV	6.1	11.6	4.9	5.1	6.2	5.9	5.9
	Mean value of Pearson's coefficient: %CV =6.52 SD=2.12							
2	1	21	94	163	187	256	296	319
	2	25	107	165	196	272	303	325
	3	20	87	135	192	231	273	301
	4	28	98	152	197	266	320	352
	5	30	110	158	200	262	314	349
	Pearson's coefficient %CV	15.6	8.5	6.9	2.3	5.5	5.4	5.8
	Mean value of Pearson's coefficient: %CV=7.14 SD =3.86							
3	1	31	87	168	227	269	308	331
	2	25	70	134	190	237	271	305
	3	29	66	123	177	218	259	297
	Pearson's coefficient %CV	8.8	12.2	13.5	10.7	8.7	7.5	4.6
	Mean value of Pearson's coefficient: %CV=9.43 SD =2.77							
4	1	8	55	107	128	163	179	215
	2	10	51	94	119	173	200	237
	3	10	48	99	123	174	204	248
	Pearson's coefficient %CV	10.1	5.6	5.3	2.9	2.9	5.6	5.8
	Mean value of Pearson's coefficient: %CV=5.46 SD =2.23							
5	1	20	88	129	198	243	290	317
	2	22	93	129	187	226	270	302
	3	17	95	138[x]	200	234	290	317
	Pearson's coefficient %CV	10.4	3.2	0.0	2.9	2.9	3.3	2.3
	Mean value of Pearson's coefficient: %CV=3.57 SD =2.98							
6	1	42	158	171[x]	207	238	275	298
	2	46	171	188	236	271	311	331
	3	56	164	188	218	248	280	298
	Pearson's coefficient %CV	12.3	3.2	0.0	5.4	5.4	5.5	5.0
	Mean value of Pearson's coefficient: %CV=5.26 SD =3.41							
Mean value: %CV		10.55	7.38	5.10	4.88	5.26	5.53	4.90
Standard deviation		2.93	3.66	4.58	2.85	1.99	1.22	1.25

[x]Explanations as in Table 7a.

T a b l e 7d. Colony forming units (CFUs) 10^6 g^{-1} fresh soil with organic fertilizers; cultured on SEA

Day of diluting and inoculating	Sample number	Time of counting colonies (days)						
		1	2	3	5	7	10	14
1	1	1	11	35	82	112	126	156
	2	5	15	45	86	119	149	185
	3	3	23	56	111	157	179	216
	4	6	23	60	114	155	180	202
	5	8	18	49	105	145	181	210
	Pearson's coefficient %CV	52.5	25.8	17.8	13.2	13.5	13.5	11.1
	Mean value of Pearson's coefficient: %CV =1.06 SD=13.61							
2	1	8	10	50	99	161	194	202
	2	8	10	51	92	152	180	198
	3	10	20	66[x]	127	197	237	251
	4	2	9	50	108	160	191	204
	5	3	5	73	90	143	173	195
	Pearson's coefficient %CV	50.4	45.9	6.6	13.0	11.3	11.4	9.9
	Mean value of Pearson's coefficient: %CV=21.21 SD =17.17							
3	1	1	3	11	65	121	164	182
	2	1	5	19	63	98	153	167
	3	3	7	21	78x	117	171	191
		2	5	15	62	98	129	151
	Pearson's coefficient %CV	47.4	28.3	23.3	9.4	1.9	10.3	8.8
	Mean value of Pearson's coefficient: %CV=18.54 SD =14.47							
4	1	4	10	35	61	94	122	132
	2	11	17	44	77	105	123	129
	3	3	7	33	68	108	125	133
	Pearson's coefficient %CV	59.3	36.9	12.8	9.5	5.9	1.0	1.3
	Mean value of Pearson's coefficient: %CV=18.10 SD =20.27							
5	1	5	7	38	78	106	137	149
	2	5	8	50	88	119	149	174
	3	5	15	48	75	101	131	156
	Pearson's coefficient %CV	0.0	35.6	11.6	6.9	6.9	5.4	6.6
	Mean value of Pearson's coefficient: %CV=10.43 SD =10.74							
6	1	3	53	122	203	259	294	314
	2	5	44	116	195	243	278	296
	3	2	50	133	196	256	301	319
	Pearson's coefficient %CV	37.4	7.6	5.7	1.8	2.7	3.3	3.2
	Mean value of Pearson's coefficient: %CV=8.81 SD =11.81							
Mean value: %CV		41.16	30.01	12.97	7.72	8.35	7.48	6.81
Standard deviation		19.53	11.93	6.12	4.66	3.59	4.53	3.54

[x]Explanations as in Table 7a.

content of the soil, as soil organic matter was assumed to be the major substrate for the growth of the bacteria (Monod, 1950). The CFUs ratio of bacteria in the soil receiving mineral fertilizer compared with the CFUs of bacteria in the soil organically fertilized was similar or the same as the ratio of organic carbon contents when the results were expressed per cm^3 of soil porosity.

The results of these studies showed that the determination of the numbers of bacteria in soil depends greatly on the method of measurement and the method of presenting the data. Moreover, as previous studies have also shown, it is useful to include the physicochemical characteristics of the soils studied (Malicki, 1980; 1981; Dąbek-Szreniawska, 1992; Stotzky *et al*., 1993), especially the physical characteristics, when interpreting the results, as this provides more information. For example, although measurements were made on fresh soil and the results presented on the basis of oven-dry soil, not providing data on the water content, bulk density, and porosity of the soils would make it difficult to compare the soils. Presenting the physical characteristics of the soils studied facilitates the comparison of microbiological data from different soils and other complex environments. Stotzky (1997) pointed out that 'by understanding the specific physicochemical factors of soils that affect positive and negative interactions, it might be possible to manipulate these factors to enhance natural biocontrol of desired and undesired microbes'. Knowledge on how soil characteristics control the activity of microorganisms in soil is essential to predict the occurrence and rate of microbially mediated functions that are of agronomic and environmental importance (Chenu and Stotzky, 2002).

CONCLUSIONS

1. The concentration of organic carbon in the soils affected the number of bacteria enumerated, as the number of CFUs was higher in the organically-fertilized soil than in the soil receiving mineral fertilizers.

2. The media used influenced the number of CFUs obtained, and determined both the time of appearance of the first colony and the kinetics of the growth of the colonies.

3. The FOR model, which requires that the colonies are repeatedly counted over a period of time, should be used to determine the potential number of bacteria in a soil and to evaluate the adequacy of the media used for the growth of the bacteria present.

4. The results obtained depend on the method of handling the soil samples, the method of measurement of growth, and how the data are expressed. In these studies, the CFUs ratio of bacteria in the minerally fertilized soil compared with the CFUs of bacteria in the soil organically fertilized was similar or the same as the ratio of organic carbon content of minerally fertilized soil compared with organic carbon content of organically fertilized soil when the results were expressed per cm^3 of soil porosity.

5. Regardless of which method is used to present the results, the physicochemical characteristics, especially the physical characteristics of the soil, must be provided. Obviously, more studies with a larger variety of soils and cultivation systems are necessary to confirm the results of this study as well as of previous studies of Hattori and co-workers.

6. It is necessary to confirm the results on determination of the numbers of bacteria in soil depends on the method of measurement and the method of presenting the data and that it is useful to include the physicochemical especially the physical characteristics of the soil.

REFERENCES

Chenu C. and Stotzky G., 2002. Interactions Between Microorganisms and Soil Particles. An overview. (Eds Huang P. M., J.-M. Bollag and N. Senesi). John Wiley & Sons, New York.

Dąbek-Szreniawska M., 1992. Results of microbiological analysis in relation to soil physical properties. Zesz. Probl. Post. Nauk Roln., 398, 1-6.

Dąbek-Szreniawska M., Sokołowska Z., Wyczółkowski A.I., Hajnos M., and Kuś J., 2002. Biological and physicochemical changes in Orthic Luvisol in relation to the cultivation system. Int. Agrophysics, 16, 15-21.

Dmitriev E.A., 1972. Mathematical Statistics in Soil Science (in Russian). Moscow Univ. Press, Moscow.

Hajnos M., Sokołowska Z., Dabek-Szreniawska M., and Kuś J., 1998. Influence of cultivation system (ecological and conventional) on porosity of a podzolic soil. Polish J. Soil Sci., 31, 33-41.

Hajnos M. and Świeboda R., 2004. Porosity and wettability of organic soilid bodies. In: Methods of the investigatios of humus substances in water and land ecosystems (Ed. D. Gołębiowska). Univ. Agric. Press Szczecin, Poland.

Hattori T., 1988. The Viable Count: Quantitative and Environmental Aspects. Science Tech. Publishers, Tokyo.

Hattori R. and Hattori T., 1980. Sensitivity to salts and organic compounds of soil bacteria isolated on diluted media. J. Gen. Appl. Microbiol., 26, 1-14.

Hattori T. and Hattori R., 2000. The Plate Count Method. An attempt to delineate the bacterial life in the microhabitat of soil. In: Soil Biochemistry (Eds J-M. Bollag and G. Stotzky) Marcel Dekker, New York.

Ishikuri S. and Hattori T., 1987. Analysis of colony forming curves of soil bacteria. Soil Sci. Plant Nutr., 33, 355-362.

Ishikuri S., Suwa Y., and Hattori T., 1984. Method for mathematical analysis of bacterial count data. Soil Sci. Plant Nutr., 30, 249-253.

Korschens M., 2004. Soil organic matter and environmental protection. Archives of Agronomy and Soil Sci., 50, 3-9.

Kuś J., 1998. Preliminary comparison of three systems of plant cultivation (conventional, integrated and ecological). Roczn. Akad. Roln. Poznań, CCCVII, Roln., 52, 119-126.

Malicki J., 1980. Physical properties of soils and their microbiological analysis. Post. Nauk. Roln., 182, 45-70.

Malicki J., 1981. Ecological base for the interpretation of the bacterial count in the soil. Roczn. Gleb., 32, 87-92.

McLean E.O., 1982. Soil pH and lime requirement. In: Methods of Soil Analysis. Part 2., (Eds A.L. Page *et al*.) ASA Inc., SSSA Inc. Madison, WI.

Monod J., 1950. The technique of continuous cultures - theory and application. Ann. Inst. Pasteur, 79, 390-410.

Parkin T.B. and Robinson J.A., 1994. Statistical treatment of microbial data. In: Methods of Soil Analysis. Part 2, (Eds R. Weaver *et al*.) SSSA Inc., Madison, WI.

Parkinson D., Gray T.R.G., and Williams S.T. (Eds), 1971. Methods for Studying the Ecology of Soil Microorganisms. Blackwell Sci. Publ., Oxford.

Snedecor G.W., 1956. Statistical Methods. Iowa State College Press, Ames, IO.

Sokołowska Z., Hajnos M., and Dąbek-Szreniawska M., 1999. Relation between absorption of water vapour, specific surface area and kind of the cultivation system. Polish J. Soil Sci., 32, 3-12.

Stotzky G., 1997. Soil as an environment for microbial life. In: Modern Soil Microbiology (Eds J.D van Elsas, E.M.H. Wellington, J.T. Trevors). Marcel Dekker Inc., New York.

Stotzky G., Broder M.W., Doyle J.D., and Jones R.A., 1993. Selected methods for the detection and assessment of ecological effects resulting from the release of genetically engineered microorganisms to the terrestrial environment. Adv. Appl. Microbiol., 38, 1-98.

Stotzky G., Goos R.D., and Timonin M.I., 1962. Microbial changes occurring in soil as a result of storage. Plant&Soil, 16, 1-19.

Tiurin I., 1931. A new modification of the volumetric method of determining soil organic matter by means of chromic acid. Pochvovedenye, 26, 36-47.