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MOBILITY OF ORGANIC PHOSPHORUS COMPOUNDS FROM PIG SLURRY IN THE SOIL

door

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ABSTRACT

10 to 20% of total P in pig slurry is contained in organic P compounds. These compounds are mainly of microbial origin in slurries stored longer than 6 months. Fresh slurries contain considerable amounts of phytate and adenosine-triphosphate, which decrease rapidly on storage. From model experiments with soil columns it follows that the bulk of organic P in pig slurry is retained in the soil-surface horizon. Only the soluble fraction of organic P, representing 5 to 10% of total organic P in pig slurry, can be very mobile in the soil. Organic P in this fraction consists mainly of high molecular weight compounds, derived from polydeoxyribonucleotides (DNA), and is only absorbed measurably in the soil at a pH below 5.

In soils to which dried pig slurry was added, between about 20% (at 2°C) and almost 100% (at 30°C) mineralisation of added organic P occurred within 12 months under static conditions. In soil columns treated with a similar quantity of dried slurry, but infiltrated with water at a rate of 1 cm/day and kept at a temperature of about 18°C, the mineralisation rate of organic P was less than could be expected from the results of the static experiment. Experimentally found rates of mineralisation and turnover of organic P in pig slurry, soil and soil treated with pig slurry were compared with the corresponding levels of phosphatase-enzyme activity. Mineralisation rates ranged from 0 to 20 mg P per week and per liter soil or slurry solution. In fresh slurry rates of up to 100 mg P per week and per liter could be found in the first week. No clear link was found with phosphatase enzyme activity.

KURZFASSUNG

10 bis 20% vom Gesammt-P in Schweinegülle kommt vor in organischen Verbindungen. Diese Verbindungen sind überwiegend mikrobieller Herkunft wenn die Gülle mehr als 6 Monate gelagert gewesen ist. In frischer Gülle gibt es grössere Mengen Inositolhexaphosphat und Adenosintriphosphat, welche während der Lagerung rasch abnehmen. Modell-Experimente mit beregneten ungesättigten Bodensäulen ergaben dass der grössere Teil des organischen P's aus Schweinegülle im Boden zurückgehalten wird. Nur der wasserlösliche Anteil des organischen P's, übereinstimmend mit etwa 5 bis 10% des Gesammt-P's der Gülle, kann sehr beweglich sein im Bodem. Dieses organisches P befindet sich überwiegend in hochmolekulären Nukleinsäure-Verbindungen. Diese Verbindungen werden nur adsorbiert bei einem pH unter 5.

In mit getrockneter Gülle behandeltem Boden wurde innerhalb etwa 12 Monate eine Mineralisation von 20% (bei 2°C) bis 100% (bei 30°C) des zugegebenen organischen P's gefunden, wenn nicht mit Wasser durchströmt wird.

In beregneten Bodensäulen (1 cm Wasser pro Tag), welche mit ähnlichen Mengen Gülle behandelt waren, war die Mineralisation etwas langsamer, wie von den Ergebnisse des statischen Experimente erwartet werden könnte.

Die Mineralisation in Gülle, Boden und mit Gülle behandeltem Boden war im Bereich von 0 bis 20 mg P pro Woche und pro Liter Gülle oder Bodenflussigkeit. In frischer Gülle selbst wurde eine Mineralisation bis etwa 100 mg P pro Woche und pro Liter während der ersten Woche gefunden. Es konnte keine Zusammenhang mit der Phosphatase-Aktivität im Boden festgestellt werden.

RÉSUMÉ

10 à 20% du P total du lisier porcin se trouve sous forme de composés organiques. Ces molécules organiques sont au cas des lisiers agés de plus de 6 mois en majeure partie d'origine microbienne. Le lisier frais contient l'hexaphosphate d'inositol et le triphosphate d'adénosine en quantités considérables mais decroissant pendant la durée du stockage. On peut conclure des expériments avec des colonnes de sol percolés avec de l'eau que la majeure partie du phosphore organique du lisier sera retenue dans la couche supérieure du sol. Seulement la partie soluble du P organique, représant dans le lisier porcin 5 à 10% du P total, peut être très mobile dans le sol. Le P organique de cette fraction a un poids moléculaire très élevé, dérivant des acides nucléiques. La sorption de ces composés macro-moléculaires ne se fait qu'à un pH infèrieure de 5.

Une minéralisation de 20% à 2°C jusqu'à presque 100% à 30°C du P organique additionné se produisit en 12 mois dans du sol incubé avec du lisier sèché. Dans des colonnes de sol percolées d'eau à 1 cm/jour pendant un an et auxquelles une quantité analogue de lisier sèché fut ajouté, la minéralisation était moins vite qu'on pourrait dduire des résultats de l'experiment au sol incubé. La minéralisation du P organique dans le lisier, le sol et le sol au lisier fut comparée avec l'activité de l'enzyme phosphatase.

La minéralisation était de l'ordre de 0 à 20 mg P par semaine et par litre de solution. Dans le cas de lisier frais on put trouver une minéralisation jusqu'à 100 mg P par semaine et par litre pendant la première semaine. Aucune liaison fut trouvé avec l'activité de l'enzyme phosphatase.

1. PHOPHORUS COMPOUNDS IN PIG SLURRY

In pig slurry 10 to 20% of total P is contained in organic P compounds. Analysis data for various P compounds in pig slurry are given in table I. In fresh slurry the organic P content can be very high (up to 1% of dry matter), mainly due to the presence of inositolhexaphosphate (IHP) and adenosinetriphosphate (ATP). Both IHP and ATP decreases rapidly and are practically negligeable after 5-6 months storage at 15-20°C. Dissolved inorganic- and organic P in pig slurry are rapidly converted into each other by the action of microorganisms. From the measurements of $^{32}PO_4$ distribution rates in pig slurry it follows that complete turn-over of dissolved inorganic P through microorganisms takes place in about 10 to 20 weeks, giving a microbial turn-over rate of about 10 mg P per week and per liter average slurry. Analysis of gel filtration fractions of pig slurry solution indicates that the dissolved organic P compounds are related to polydeoxyribonucleotides (DNA) and have a high molecular weight. Mineralisation rates of organic P in pig slurry can vary greatly and are influenced by aeration, storage time, dry matter content and temperature. Results of following the organic P contents of various pig slurries with storage time are shown in table II. The concentration of dissolved organic P was in all cases unaffected by storage time and remained constant at 10 to 20 mg P per liter. From table II it can be said that for pig slurry stored between 1 week and 1 year, mineralisation rates of organic P will lie between 0 and 20 mg P per week and per liter slurry, increasing with decreasing aeration. The corresponding rates for loss of organic matter are 3 to 0.1 g per week and per liter slurry, decreasing with decreasing aeration.

TABLE I. Distribution of phosphorus among organic and inorganic phosphorus compounds in pig slurry.
10 slurries were analysed. Dry matter content ranged from 5 to 10% and time of storage from 1 to 6 months.

	P content: (% of dry matter)	Average of total P:
total inorganic P	1.5 - 2	85 %
total organic P	0.2 - 0.3	15
inorganic P in solution	0.01 - 0.2	5
P in inositol hexaphosphates (IHP)	0.01 - 0.1	2
P in microorganisms	0.02 - 0.04	1.5
P in adenosine-5-triphosphate (ATP)	0.000 - 0.1	1
organic P in solution	0.01 - 0.03	1

TABLE II. Organic phosphorus (P_{org}) and dry matter (DM) contents of pig slurry as a function of time of storage.
Pig slurries 1 and 2 were stored in closed containers, relieving pressure when necessary. Slurry 3 was stored in a wide-necked open container. Slurry 4, identical to slurry 3, was aerated and agitated during storage. Data are related to 1 kg slurry at time = 0.

Storage time: (weeks)	Slurry 1		Slurry 2		Slurry 3		Slurry 4	
	DM (g)	P_{org} (mg)	DM (g)	P_{org} (mg)	DM (g)	P_{org} (mg)	DM (g)	P_{org} (mg)
0	74	280	63.5†	400	-	-	-	-
1	73	210	-	-	68	160	66	200
3	-	-	-	-	67	150	59.5	200
6	67	130	-	-	-	-	-	-
7	-	-	-	-	62.6	150	51.2	200
10	-	-	-	-	60.5	140	39.9	200
16	-	-	-	-	55.3	110	-	-
18	63.8	80	-	-	-	-	-	-
21	61.5	80	-	-	-	-	-	-
63	-	-	57.7††	75	-	-	-	-
132	34.7†††	70	-	-	-	-	-	-

† organic matter = 73.2% (of DM)
†† organic matter = 65.8%
††† organic matter = 60.9%

2. SOIL FIXATION

Pig production in the Netherlands is particularly intensive in the sandy soil regions. Pig waste in the form of slurry with a dry matter content of 5 to 10% is sometimes dumped on soils at rates of 100 and more tons per ha and per year. Under average conditions a strong fixation of inorganic P in soils can be expected. The main factors affecting this fixation appear to be: Fe, Al and Ca content of the soil, specific surface area, pH and redox potential. Time is also an important parameter. Fixation of inorganic P in soils increases after an initial rapid adsorption slowly with time due to chemical immobilisation reactions with rate constants of the order of $(months)^{-1}$ to $(years)^{-1}$. The rate of adsorption of inorganic P from pig slurry solution in sandy soils has a maximum at a pH of about 5 (fig. 1).

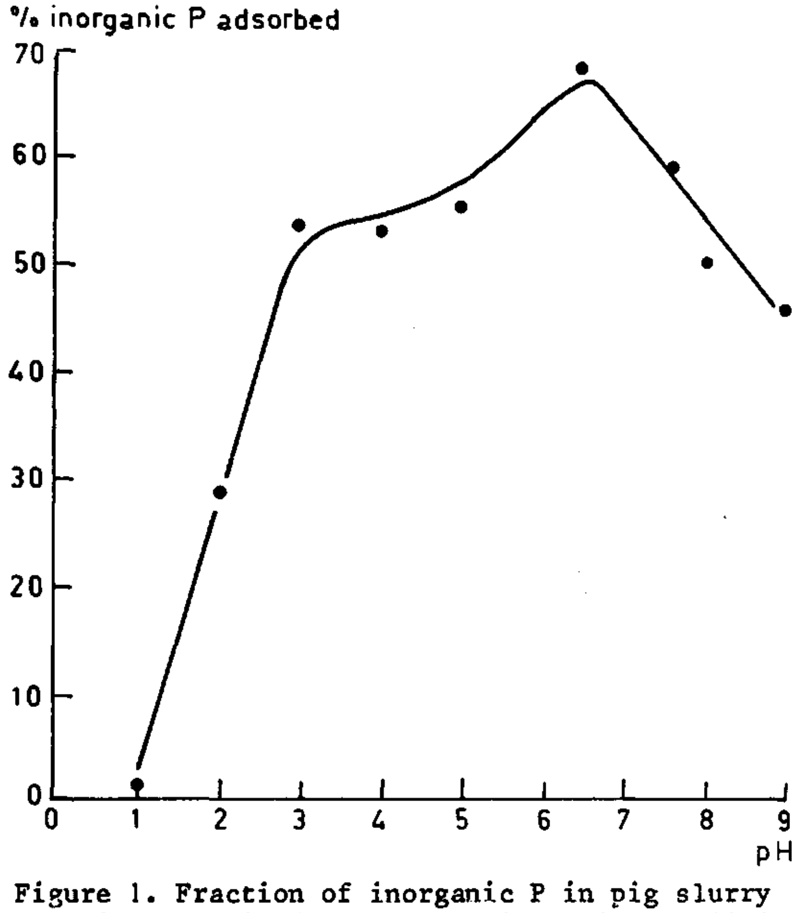

Figure 1. Fraction of inorganic P in pig slurry solution, obtained after centrifugation at 40.000 G for 30', adsorbed to soil after 24h. 10 g soil was equilibrated with 40 ml slurry solution at various pH.

The adsorption was measured after 24h equilibration of 10 g soil with 40 ml slurry solution. Apparently the $H_2PO_4^-$ ion of which the concentration is at a maximum at pH 5 is the rate determining ion. Organic P dissolved in pig slurry is only adsorbed measurably in sandy soils below a pH of about 5 (fig. 2). Organic P in pig slurry solution can thus be much more mobile in the soil than inorganic P. Organic P forms an important part of total soil P. In the surface horizon of soils 10 to 80% of total P is contained in organic compounds. A larger part of this soil organic P can be in phytates, which are Fe, Al, Ca and Mg complexes of myo-inositol hexaphosphate (IHP).

Also high molecular weight complexes containing IHP and other organic P compounds have been found in soil solution. Most organic P compounds in the soil can be utilised by plants and microorganisms either directly or after degradation. Phytates however, especially Fe and Al phytate, are very persistent in the soil and almost unavailable to plants and microorganisms.

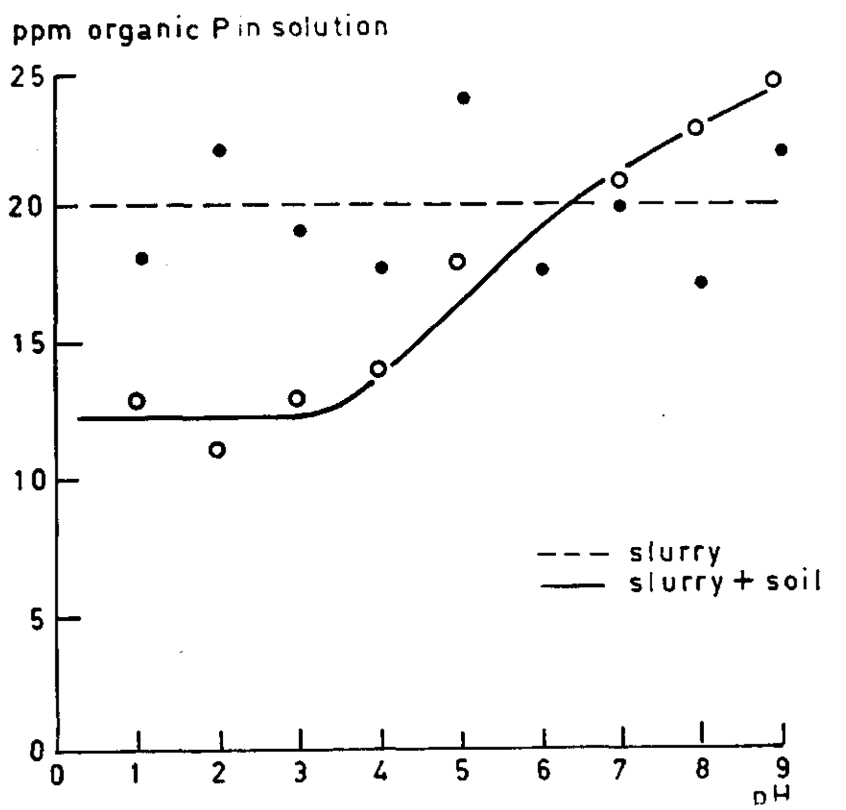

Figure 2. Adsorption of organic P from pig slurry solution to soil under the same conditions as given in Fig. 1.

In table III results are shown of the calculation of some possible contributions to the IHP content of a sandy soil. A steady increase of soil phytate can be expected if it is assumed that all IHP contributed is bound and only slowly decomposed. The contribution of soil microorganisms to phytate P is difficult to estimate. Sometimes e.g. in the case of Phragmites (common reed) large amounts of IHP are produced in the rhizosphere under eutrophic conditions.

TABLE III. Phytate (IHP) content of the surface horizon of a podzol soil and some possible sources of IHP accumulation. Total amounts of inorganic and organic P involved are also given.

	P_{IHP}	$P_{inorganic}$	$P_{organic}$
soil layer (0-20 cm)	150 kg/ha	900 kg/ha	600 kg/ha
100 tons of pig slurry per ha	0.5 - 10	75 - 200	10 - 30
dead grass roots per year	0.2 - 0.65	1.9 - 6.2	1.6 - 5.2
dead worms per year	0.01 - 0.2	0.2 - 3.5	0.5 - 6.7

3. DECOMPOSITION OF ORGANIC P UNDER STATIC CONDITIONS

This was investigated in the following experiment. Three batches of 1 kg of the surface horizon of a reclaimed podzol were air dried and mixed with 50 g freeze-dried pig slurry (roughly equivalent to a treatment of 500 to 1000 tons pig slurry per ha). Water was then added until the pF of the soil + slurry mixture was about 2. The resulting batches of soil + slurry were then incubated for twelve months, each batch at a different temperature (2, 20 and 30°C). The water content was kept constant. Three batches of the same soil were treated with the neutral sodium salt of IHP and kept under the same conditions as the slurry treated soils. In the same way three batches of untreated soils were used as reference. Changes in P, N, organic matter and pH are shown in tables IV a, b and c. At 30°C organic P from pig slurry added to the soil appears to be almost completely mineralised after 1 year, while at 20°C roughly 40% and at 2°C roughly 20% is mineralised after one year. IHP, when added as neutral salt, is apparently not decomposed at all under the conditions of the incubation experiment. In the untreated soil the decomposition of organic P is in the range of a few percent, amounting about 1 mg P per liter soil solution per week. Phosphatase activities were also measured in the incubated batches of soil from the decomposition rate of added p-nitrophenylphosphate. Michaelis-Menten kinetics applied reasonably well. Results are given in table V. Only in the case of pig slurry after about 30 weeks the enzyme concentration (proportional to V_{max}) starts to increase. In all other cases K_m and V_{max} do not appear to change significantly, though with pig slurry the K_m is significantly greater, pointing to an inhibition effect.

TABLE IV a. Results of the analysis of a sandy top soil incubated at various temperatures for twelve months. The pF of the soil was kept between 1 and 2.

Time (weeks)	T °C	P_{inorg} %	P_{org} %	Organic matter %	$pH(H_2O)$	$N_{Kjeldahl}$ %
4	2	0.033	0.028	6.0		0.17
4	20	0.033	0.028	6.0		0.16
4	30	0.034	0.026	6.0		0.16
13	2	0.035	0.027	6.1		0.16
13	20	0.035	0.027	5.9		0.16
13	30	0.035	0.028	6.0		0.15
30	2	0.035	0.024	6.2	4.6	0.16
30	20	0.034	0.027	6.1	4.5	0.16
30	30	0.035	0.023	5.9	4.6	0.16
54	2	0.034	0.026	6.2	4.6	0.15
54	20	0.035	0.026	5.9	4.5	0.15
54	30	0.036	0.026	5.7	4.6	0.14

TABLE IV b. Results of the analysis of a sandy top soil incubated at various temperatures after mixing with pig slurry. The pF of the soil was kept between 1 and 2.

Time (weeks)	T °C	P_{inorg} %	P_{org} %	Organic matter %	$N^{\dagger}_{Kjeldahl}$ %	N_{NO_3} %	N_{NH_4} %	$pH(H_2O)$
4	2	0.094	0.04	9.6	0.31	0.005	0.05	
4	20	0.095	0.04	8.6	0.29	0.055	0.01	
4	30	0.094	0.04	8.2	0.28	0.041	0.025	
13	2	0.096	0.04	8.7	0.30	0.019	0.05	
13	20	0.102	0.035	8.3	0.26	0.076	0.001	
13	30	0.105	0.03	7.8	0.25	0.056	0.01	
30	2	0.095	0.045	8.9	0.26	0.060	0.015	5.0
30	20	0.100	0.04	8.3	0.23	0.079	0.000	4.5
30	30	0.106	0.03	7.6	0.20	0.068	0.005	4.8
54	2	0.097	0.04	8.6	0.26	0.062	0.012	5.0
54	20	0.099	0.04	7.8	0.25	0.081	0.001	4.45
54	30	0.107	0.03	7.0	0.20	0.066	0.017	5.0

† Kjeldahl determinations were done without addition of salicylic acid, thus giving unknown interference by NO_3.

TABLE IV c. Results of the analysis of a sandy top soil incubated at various temperatures after mixing with inositol hexaphosphate. The pF of the soil was kept between 1 and 2.

Time (weeks)	T °C	P_{inorg} %	P_{org} %	Organic matter %	$pH(H_2O)$	$N_{Kjeldahl}$ %
4	2	0.027	0.120	6.3		0.17
4	20	0.029	0.120	6.2		0.15
4	30	0.028	0.120	6.2		0.17
13	2	0.029	0.115	6.3		0.16
13	20	0.028	0.120	6.3		0.14
13	30	0.028	0.120	6.3		0.16
30	2	0.029	0.120	6.3		0.17
30	20	0.030	0.130	6.1		0.16
30	30	0.028	0.125	6.2		0.16
54	2	0.028	0.125	6.2	5.8	0.16
54	20	0.027	0.127	6.0	5.5	0.15
54	30	0.029	0.128	6.0	5.4	0.14

TABLE V. Michaelis-Menten rate constants of acid phosphatase activities of a sandy top soil incubated at various temperatures for twelve months with and without addition of inositol hexaphosphate or pig slurry. The pF of the soils was kept between 1 and 2.

K_m in µmoles per liter; V_{max} in µmoles per liter per gram per hour.

A	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
	4 weeks		13 weeks		30 weeks		54 weeks	
soil:								
2°C	70	110	50	80	60	75	80	85
20	50	100	75	80	65	70	80	85
30	60	90	100	80	80	90	80	95
Soil + phytate:								
2°C	60	100	90	90	100	80	50	75
20	60	85	70	85	70	75	75	90
30	40	70	80	60	70	75	70	85
soil + slurry:								
2°C	90	130	100	85	100	95	160	150
20	100	110	90	90	160	220	105	235
30	90	75	105	90	145	200	180	370

After 54 weeks the soils were sampled and equilibrated with water in the ratio 45 g soil to 60 ml water for 24h. After centrifugation at 40.000 G during 30' the supernatant was analysed for organic and inorganic P. As can be seen from the results in table VI enormous differences in water extractability of P are caused by the various treatments. The low concentrations of organic P in the case of soil+slurry coincide with a high level of enzyme activity. The high concentration of inorganic P, both in the case of soil+slurry and in the case of soil+IHP, is most likely due to competitive adsorption with subsequent displacement of inorganic P. The high concentration of organic P in the case of soil+IHP is also probably due to displaced soil organic P.

TABLE VI. P analyses of the solutions obtained after shaking the sandy top soils incubated with inositol hexaphosphate and pig slurry with water.
A ratio of 45 g soil to 60 ml water was used. The solution was centrifuged at 40.000 G for 30'. The analyses were done after 54 weeks of incubation.

	Total P mg/liter	Inorganic P mg/liter	Organic P mg/liter
Soil:			
2°C	0.188	0.122	0.066
20	0.145	0.109	0.036
30	0.226	0.106	0.120
Soil + phytate:			
2°C	9.44	5.22	4.22
20	5.69	3.18	2.51
30	4.11	2.61	1.50
Soil + slurry:			
2°C	11.35	10.4	0.95
20	7.66	7.65	0.01
30	4.60	4.60	0.00

4. DECOMPOSITION OF ORGANIC P UNDER DYNAMIC CONDITIONS

Sandy soils were cored to a depth of 100 cm in cylindrical perspex tubes (cross sectional area 113 cm^2). The top 10 cm of the soil column was mixed with 70 g freeze-dried pig slurry (equivalent to a treatment of about 1000 tons per ha). Rainfall was simulated at a rate of 1 cm per day such that water flow through the column was at equilibrium. The column effluent was monitored for inorganic and organic P, N, pH, chloride and phosphatase activity. After a year the percolation was stopped and the soil analysed in layers. Some effluent data of a podzol soil column are shown in fig. 3. In another experiment a similar soil was treated with 1 liter of pig slurry solution, obtained after centrifugation at 40.000 G, at a rate of 1 cm per day and subsequently percolated with water in the same way and at the same rate as was done for the column treated with dried slurry. Effluent data are given in fig. 4 As can be seen from figs. 3 and 4 the only mobile P appears to be dissolved organic P, in spite of the heavy loadings. Organic P in solution in pig slurry can be expected as was mentioned before to remain completely mobile at pH's above 5. Indeed high concentrations of organic P in the effluent coincide with a pH of the effluent well above 5. This influence of the pH probably also explains the "double" peak observed for organic P in the effluent of the soil column treated with freeze-dried slurry (fig. 3). Molecular weight characteristics of organic P in the column effluents were similar to those of organic P in pig slurry. Also (table VII) the total amount of organic P eluted from the soil columns is roughly equal to what can be expected from the amount of pig slurry or slurry solution brought into the column. In fig. 5 results are given of the analysis of the profile of the soil columns for inorganic and organic P. The distance over which inorganic and organic P have penetrated into the soil after one year under the conditions of the experiment is about 5-15 cm. An input-output balance of P and N in the soil columns was made and is given in table VII. From the nitrogen data it follows that microbial action in the form of mineralisation and denitrification is well developed.

The amount of organic N mineralised is considerably more than the amount added, so the addition of slurry seems to have had a strong priming effect, particularly in the case where slurry solution is added. This is not in agreement with practical experience where on average 70% of the organic N from manure is mineralised in the first year. According to table VII denitrification is high in both cases and relatively highest where slurry solution is added. Mineralisation of organic P appears to be less than can be expected from the experiment under static conditions. In the soil column treated with slurry solution even a net synthesis of organic P seems to occur. Apparently the N/P ratio is too high there. A high level of phosphatase activity in the effluents of the soil columns coincided with the peak of organic P. Apart from this peak in phosphatase activity, levels were almost zero, so that it is unlikely that microorganisms are transported through the column. Calculating the range of pore widths through which water flow occurs at values of pF between 1 and 2 gives an indication of what size objects can migrate into the

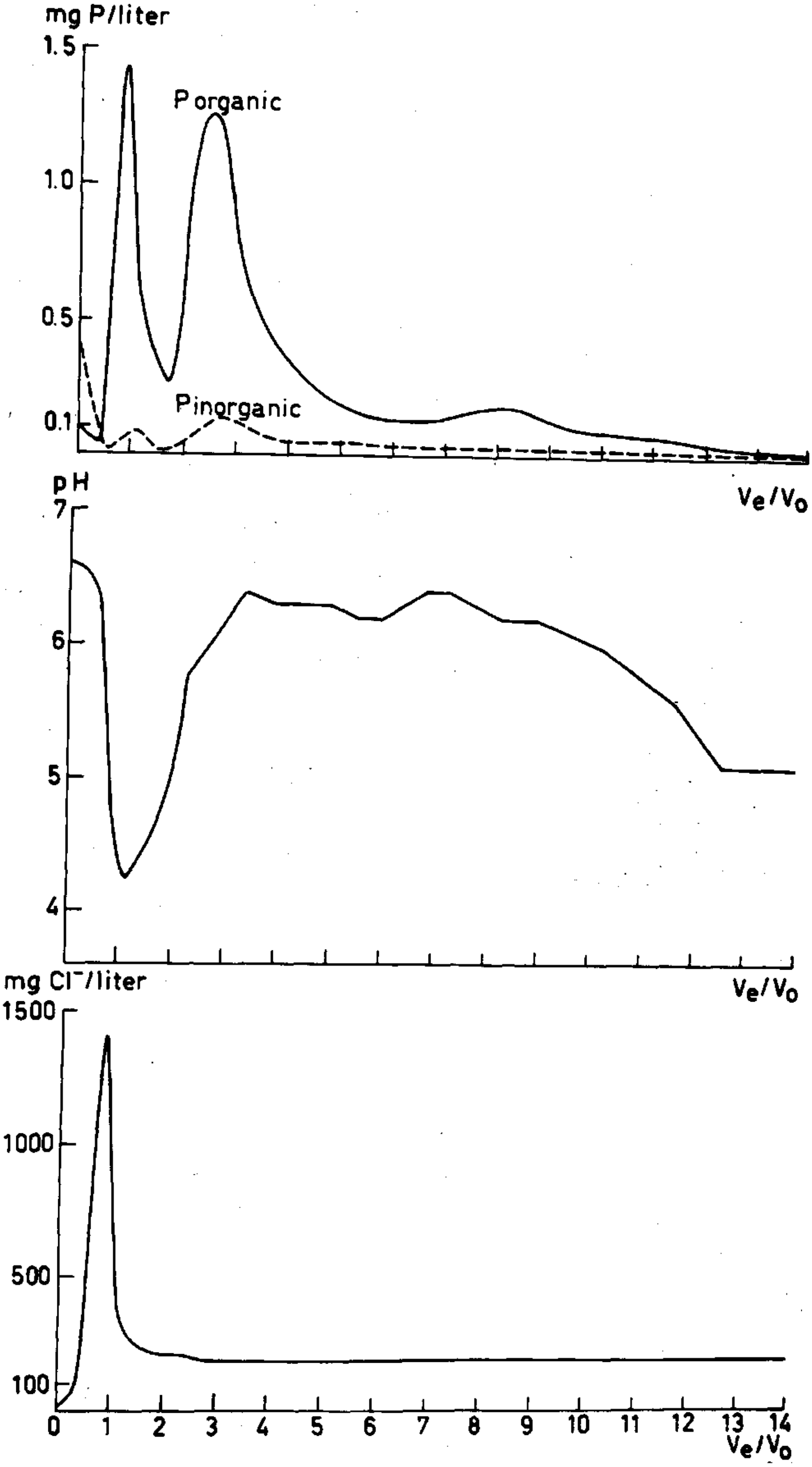

Figure 3. Effluent data for a reclaimed "haar" podzol soil column. Rainfall in the column was simulated at a rate of 1 cm per day. Volumetric water content of the column (V_o) was 2.8 liter. The top 10 cm of the column was mixed with dried pig slurry (70 g).

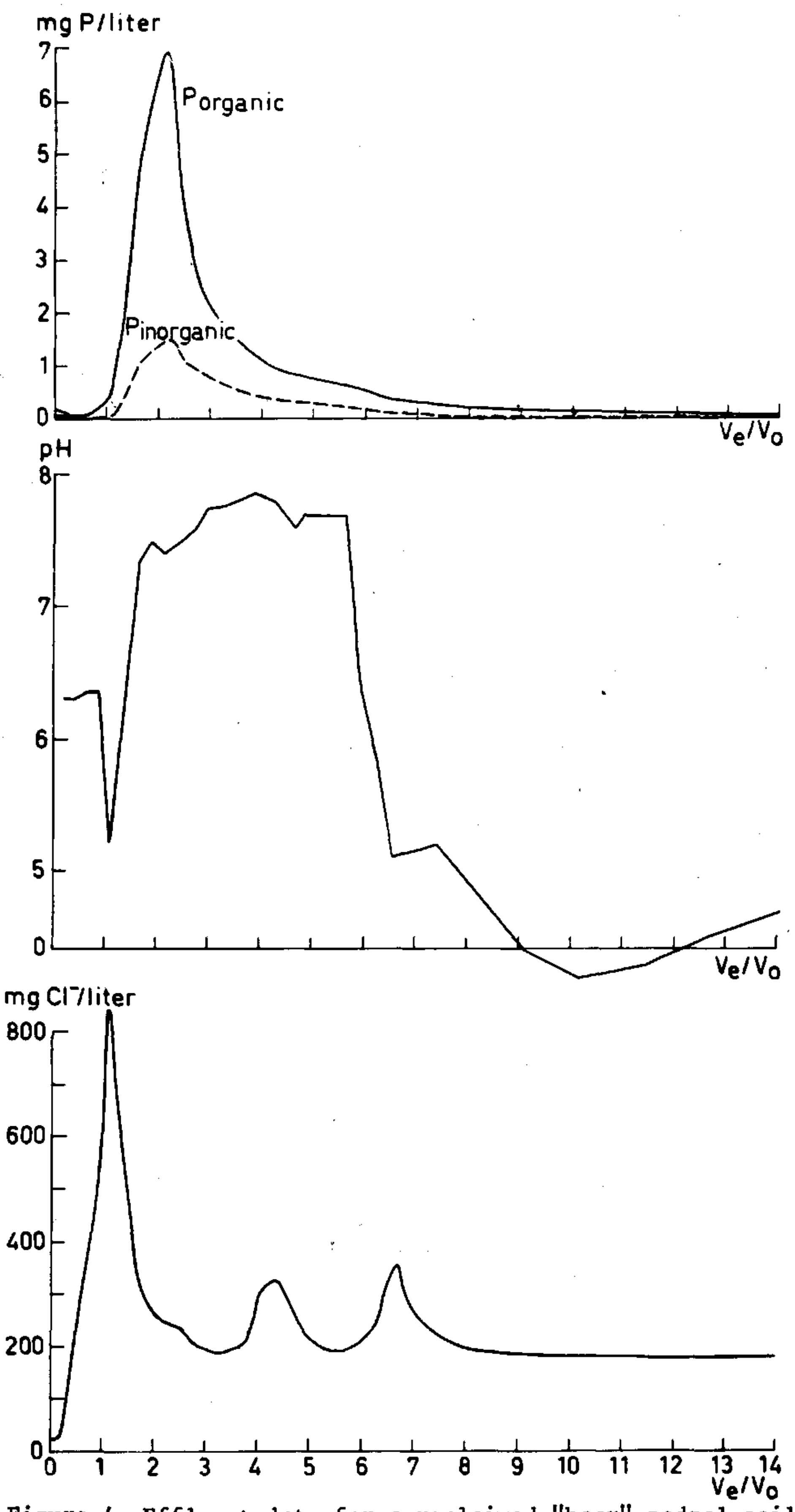

Figure 4. Effluent data for a reclaimed "haar" podzol soil column. The column was treated with 1 liter of slurry centrifugate instead of dried pig slurry. Other conditions as in Fig. 3.

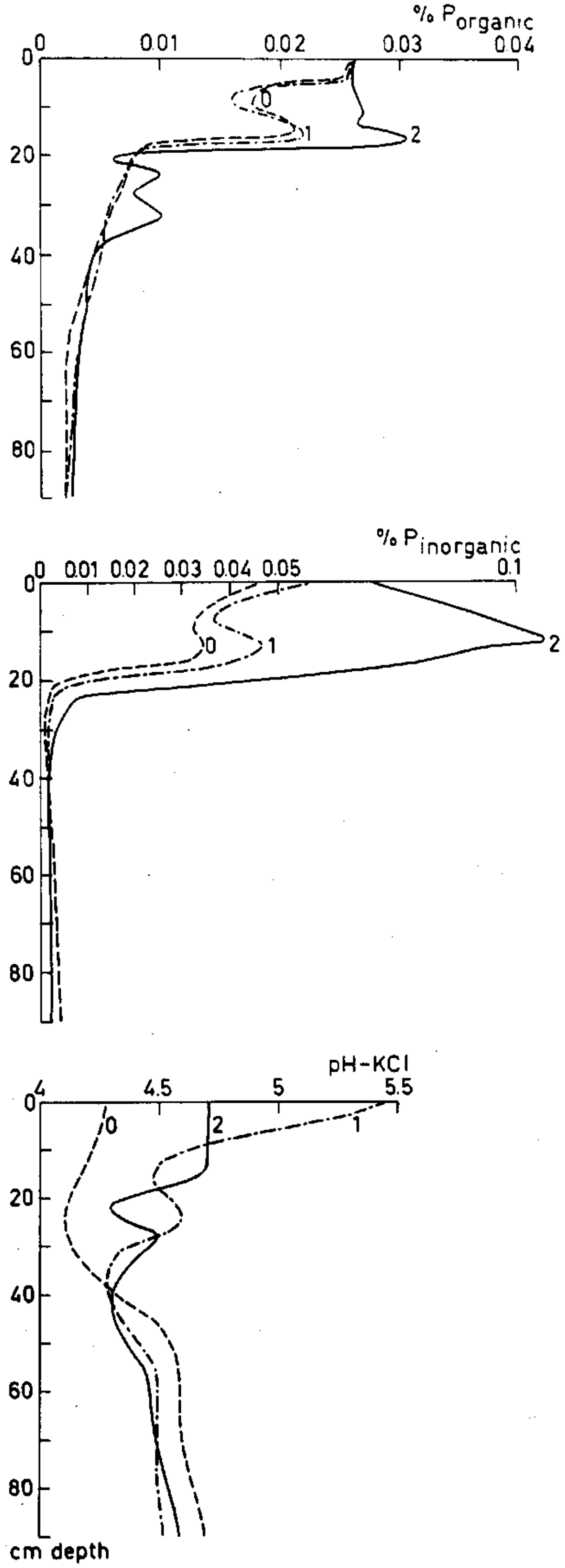

Figure 5. Results of analyses of soil columns of a reclaimed "haar" podzol without (0), and with addition of dried pig slurry (2) or slurry centrifugate (1), after 1 year (total effluent volume = 39.5 liter).

soil profile. For the sandy soil used an upper (exclusion) limit of 10-100 μm was calculated, giving considerable obstruction of the movement of bacteria. Migration of viruses under these conditions is possible as is indeed confirmed by the literature. Viruses appear to be mobile in the soil at a pH above 7, while below 7 they become increasingly adsorbed. This phenomenon is somewhat analogous to the behaviour of organic P dissolved in pig slurry in the soil.

TABLE VII. Input-output balance for phosphorus and nitrogen in the soil columns treated with pig slurry or slurry centrifugate and percolated with water at a rate of 1 cm per day. The columns were analysed after 1 year.

	$P_{inorganic}$ (mg)	$P_{organic}$ (mg)	$N_{organic}$ (mg)	N_{NO_3} (mg)	N_{NH_4} (mg)
total content in:					
soil column + dried pig slurry	2670	1215	7220	42	91
soil column + slurry centrifugate	1535	1045	6600	31	10
soil column --- initial situation	1450	1010	8430	53	8
cumulative content in the effluent of:					
soil column + dried pig slurry	1.4	10.3	520	1180	1900
soil column + slurry centrifugate	8.6	31.9	670	1620	3550
Content of:					
added pig slurry	1180	250	1690	0	1420
added slurry centrif.	170	20	350	0	4320
balance of:					
soil column + dried pig slurry	+40	-35	-2380	+1170	+560
soil column + slurry centrifugate	-76	+47	-1510	+1600	-770

5. Literature:

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