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Dissolved organic and inorganic phosphorus compounds in pig slurry: effect of drying

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SUMMARY

From gel filtration studies it has been found that more than 50% of organic phosphorus dissolved in pig slurry is contained in compounds of high molecular weight. Various ions, e.g. calcium, copper, orthophosphate, are bound by these compounds. From the purine and pyrimidine base composition and resistance to acid and alkali treatment it follows that these organic compounds probably are complexes derived from polydeoxyribonucleotides (DNA).

The effect of drying pig slurry at various temperatures (0–100 °C) on the solubility of phosphorus, calcium and copper after redispersion of the dried slurry was investigated. The solubility of organic phosphorus was not affected by drying and redispersion in water, but the amount of phosphorus contained in dissolved organic molecules of high molecular weight decreased on drying at higher temperatures. The solubility of copper was also not affected by heat treatment. The solubility of inorganic phosphorus is mainly related to the solubility constants of mineral phosphates. On the other hand the total solubility of the cations involved is determined by complex formation.

INTRODUCTION

The storage capacity of a soil for inorganic phosphorus (as phosphate), estimated roughly from its adsorption isotherm (Powers, Kani & Zinke, 1975) and rate of immobilization of inorganic phosphorus (Larsen, Gunary & Sutton, 1965), is known to be large. Not much is known, however, about the storage capacity of a soil for organic phosphorus. There are indications that certain organic phosphorus compounds are leached from the soil very easily (Hannapel, Fuller & Bosma, 1963; Rolston, Rauschkolb & Hoffmann, 1975; Campbell & Racz, 1975). As part of a study of the filter capacity of soil for phosphorus from pig slurry it was thought important, in this context, to establish the nature of phosphorus compounds present in solution in pig slurry. In previous papers (Gerritse & Zugec, 1977; Gerritse, 1977) mention was made of the presence of dissolved organic phosphates of high molecular weight in pig slurry. Microbiological aspects were discussed and it was supposed that these organic phosphates consisted of complexes of DNA with (poly) phosphates and certain cations such as calcium and, if used in the feed, copper. Substantiation of this assumption was however necessary.

As copper is still used in pig feeds in the Netherlands and the copper content of slurries is about 100–1000 μg/g (dry matter) it was thought interesting to establish the nature of dissolved copper compounds, especially as they might be complexed to organic phosphorus compounds. These organic phosphorus compounds may also play an important part in the transport of copper and other heavy metals in the soil (Lexmond & De Haan, 1977).

EXPERIMENTAL PROCEDURE

The fractionation of dissolved organic phosphorus compounds in pig slurry by gel filtration through Sephadex G-100 (Pharmacia) followed by subsequent analyses of total, inorganic and organic phosphorus has been described in a previous paper (Gerritse & Zugec, 1977).

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Copper, calcium and magnesium were analysed by atomic absorption spectrophotometry after destruction with HNO_3/HF (8:1 by volume) and addition of $SrCl_2$ (H. C. Vierveijzer, personal communication). Iron and aluminium were determined colorimetrically according to standard methods with orthophenantrolin and xylenolorange, respectively (H. A. Sissingh, personal communication).

Purine and pyrimidine bases were analysed by high speed high pressure liquid chromatography (HPLC) using an anion exchanger (Aminex A-28, Bio-Rad Laboratories) as stationary phase and eluting with a 1:1 (by volume) mixture of ethanol and water, containing 0·005 M sodium citrate and 0·05 M phosphate ($KH_2PO_4 + K_2HPO_4$), buffered at a pH of 7·25 (measured before adding ethanol). Citrate acts as the main counter ion of the ion exchanger. The ion exchanger was used at a temperature of 70 °C. The chromatographic system consisted of: a thermostatted elutant reservoir, maintained at a temperature 5 °C above column temperature and fitted with a reflux condenser; a reciprocating high pressure liquid pump (Orlita MK-00); a Bourdon type pressure gauge and capillary restrictor connected in series with the pump, in order to dampen pump pulsations (Huber, 1969); an air thermostat (Siemens) for the column; a six-port sampling valve (Valco), with a 13·6 µl injection volume; a spectrophotometer (Zeiss PMQ-2) modified to accommodate a micro flow cell (volume 8 µl; pathlength 1 cm) (all analyses were carried out at a wavelength of 260 nm and a slit-width of 2 mm); a recorder (Servogor RE 511), and a column (length 25 cm, internal diameter 0·29 cm, external diameter 0·6 cm) and connecting tubing of stainless steel (SS 316) and low volume connexions (Swagelock).

The separating column was packed under working conditions with a suspension of the ion exchanger at a column pressure of 80 bar. After packing, the column was equilibrated for 3 h, maintaining the pressure at 80 bar.

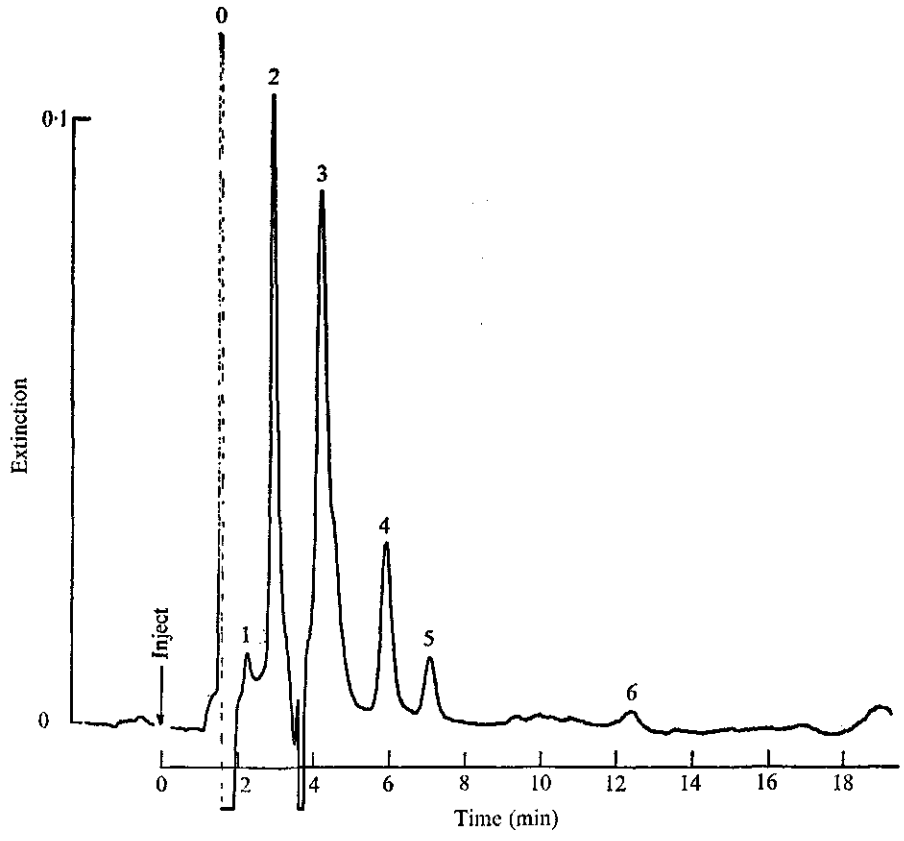

Fig. 1. High pressure liquid chromatogram of a hydrolysate of the high molecular weight gel filtration fraction of pig slurry. The identified peaks are: (1) cytosine, (2) thymine, (3) adenine, (4) guanine, (5) hypoxanthine, and (6) xanthine.

Gel filtration fractions obtained from 5 ml of pig slurry were evaporated at 100 °C to a volume of 1 ml, transferred to a test tube and heated with 0·5 ml of 12 N-$HClO_4$ at 100 °C for 2 h. After this the mixture was cooled and treated with 1 ml 8 N-KOH at 0 °C and kept at 0 °C for 30 min (Benditch, 1957; Gehrke & Ruyle, 1968). After centrifuging, the supernatant was decanted from the precipitate and the precipitate washed twice with water of 0 °C. The resulting solution was used for chromatographic analysis of the purine and pyrimidine bases after evaporation to a (measured) volume of 3–5 ml.

RESULTS AND DISCUSSION

Identification of high molecular weight phosphorus

In Fig. 1 a chromatogram is shown of the bases obtained after hydrolysis of the high molecular weight gel filtration fraction of pig slurry solution. In Fig. 2 a chromatogram of the hydrolysate of the low molecular weight fraction is shown. The peaks in the chromatograms were identified by determining the capacity ratio with respect to the salt peak (0 in the Figs.) and comparing them with the values obtained for the pure bases. A chromatogram of a mixture of the pure bases is given in Fig. 3. The capacity ratio is calculated from the ratio of the net retention time of a peak (total retention time minus retention time of the salt peak) and the retention time of the salt peak, assumed to represent the retention time of a non-retarded component. The entire procedure was tested with calf thymus DNA of known composition, of which a chromatogram is shown in Fig. 4. From Figs. 1 and 2 it is evident that the high molecular weight fraction contains much more of the bases than the low molecular weight fractions. Cytosine, thymine, adenine, guanine, hypoxanthine and xanthine were positively identified, while uracil was not found. The thymine and adenine peaks in the chromatograms of the hydrolysates are partly overlapped by unidentified peaks of what are probably other bases. In Table 1 average results are given of the quantitative analysis of a number of samples of fractionated pig slurry solution. In

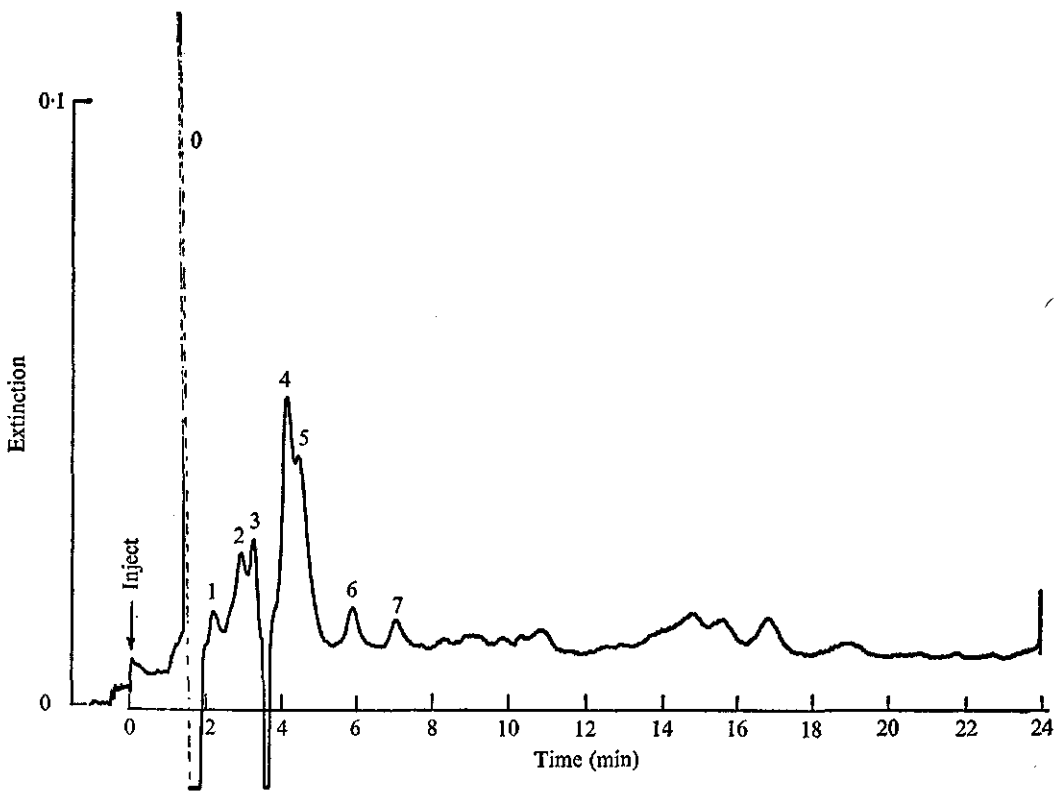

Fig. 2. High pressure liquid chromatogram of a hydrolysate of the combined intermediate and low molecular weight gel filtration fractions of pig slurry. The identified peaks are: (1) cytosine, (2) thymine, (3) ?, (4) adenine, (5) ?, (6) guanine, (7) hypoxanthine.

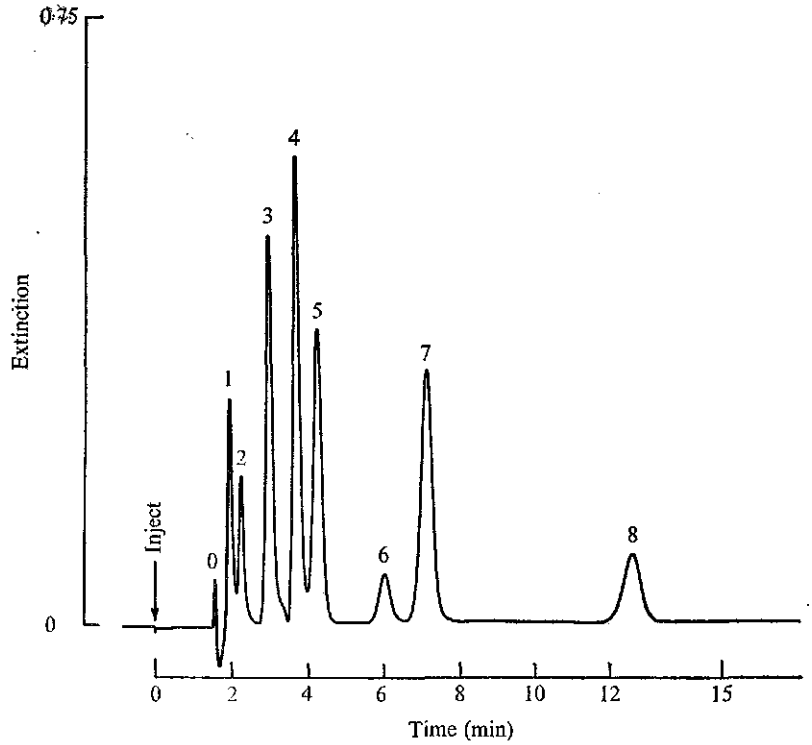

Fig. 3. High pressure liquid chromatogram of a mixture of bases: (1) 5-methyl cytosine, (2) cytosine, (3) thymine, (4) uracil, (5) adenine, (6) guanine, (7) hypoxanthine, (8) xanthine.

view of the presence of thymine and the absence of uracil it can be assumed that the polymeric compounds in the high molecular weight fraction of pig slurry solution are derived from DNA. The base composition found, however, is unusual for normal DNA and especially the low cytosine content is odd. The total concentration of bases in these high molecular weight compounds amounts to about 200–300 μmole/l, which assuming a DNA structure, gives 6–9 μg P/ml. This accounts for about half of the total amount of P in the high molecular weight fraction (Table 2). No directly comparable results have been found in the literature, though Minear (1972) states that of the high molecular weight phosphorus compounds occurring in lake water 40–60 % of the phosphorus is accounted for by DNA.

Inorganic counter ions of the phosphorus compounds dissolved in pig slurry. Effect of drying at different temperatures

In Table 2 total P, inorganic P, Cu and Ca are given for various gel filtration fractions of pig slurry solution. Organic P is taken as the difference between total P and inorganic P. Analyses were done with fresh slurry, freeze-dried slurry and slurry dried at 60 and 100 °C. Before analysis the dried and ground slurry material was resuspended in water and shaken gently for 24 h. The slurries were made up to the same dry-matter content as before drying. Analysis of five other pig slurries treated in the same way gave results similar to those in Table 2. The slurries were between 2 and 6 months old and were from pigs weighing about 60–70 kg. Ca/P ratios in the feed varied as did the absolute quantities in the slurries of the minerals investigated, especially Ca and P (Gerritse & Zugec, 1977). The results are given as typical of this series of samples, taken to be average type slurry. Effects such as age of the animals, feed composition, and conditions of storage of the slurries were not investigated. As can be seen, about a quarter of the Ca and Cu and more than half of the organic P in solution in pig slurry is found in the high molecular weight fraction of untreated or freeze-dried slurry. The concentration of dissolved organic phosphorus compounds of high molecular weight appears to decrease after drying at 60 and 100 °C and subsequent rewetting,

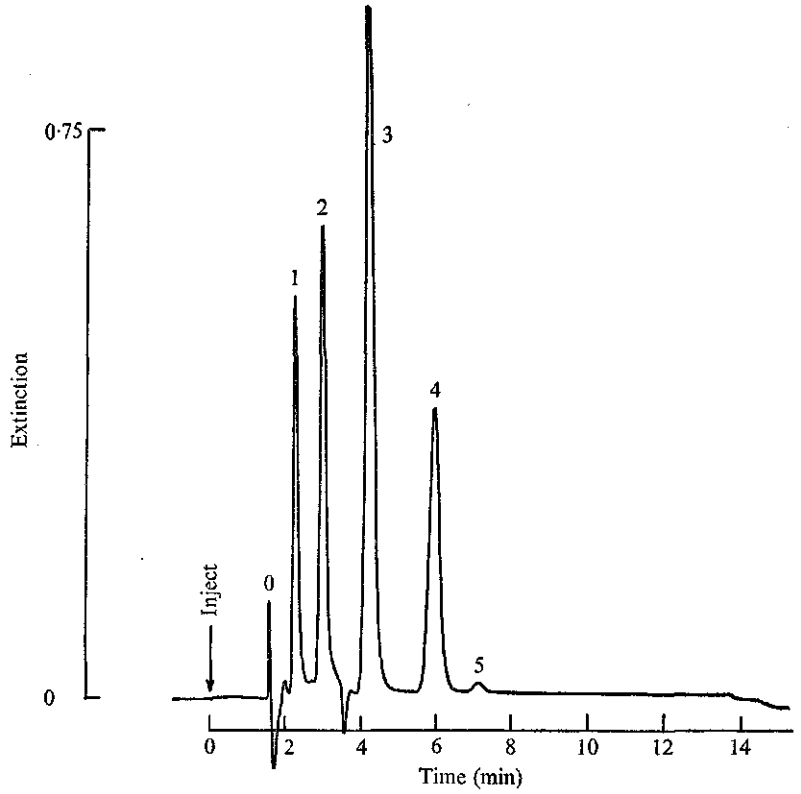

Fig. 4. High pressure liquid chromatogram of a hydrolysate of DNA from calf thymus: (1) cytosine, (2) thymine, (3) adenine, (4) guanine, (5) hypoxanthine.

Table 1. *Results of the analyses of purine and pyrimidine bases in gel filtration fractions of pig slurry solution after hydrolysis and chromatographic separation*

	High molecular weight fraction (μmole/l)	Low + intermediate molecular weight fraction (μmole/l)
Cytosine	5	3
Thymine	50	5
Adenine	35	10
Guanine	35	7
Hypoxanthine	15	7
Xanthine	10	—

The coefficient of variation was about 5–10 %. Overlapping peaks were quantified approximately by using the peak areas after section at the minimum between adjacent peaks.

while total organic P does not seem to alter significantly. The calcium content of the high molecular weight fraction appears to decrease proportionally with organic P content, while copper content remains constant.

The solubility of inorganic P is influenced mainly by pH and calcium concentration in the slurry. As part of the calcium and phosphate dissolved in pig slurry is present in complexes it is not possible to calculate a solubility product from direct measurements of P and Ca in pig slurry solution. If $CaHPO_4 . 2H_2O$ is the principal mineral phosphate present in slurry (Barrow, 1975) the solubility product can be calculated from:

$$K = \alpha_{Ca} . [Ca^{2+}] . \alpha_P . [HPO_4^{2-}], \qquad (1)$$

where α is the activity coefficient and brackets denote concentration.

Putting the ionic strength at 0·1 in all cases, the product $\alpha_{Ca} . \alpha_P$ can be calculated to be about 0·15 from the extended Debye–Hückel equation (Kielland, 1937). With equation (1) and the data from Table 2 for Ca and P in the low molecular weight fractions, the corresponding solubility constants were calculated. The calculation assumed negligible complex formation in the low molecular weight fraction and also that all of the inorganic P was present as orthophosphate. Thus, the data shown in Table 3 give an average pK value of $6·2 \pm 0·3$ at a temperature of 20–25 °C. The solubility product

Table 2. *Results of the analysis of gel filtration fractions of untreated (fresh) and treated (dried and resuspended) pig slurry*

Description	pH	P_{total} (μg)	$P_{inorganic}$ (μg)	$P_{organic}$ (μg)	Cu (μg)	Ca (μg)
Untreated	8·60	390	370	20	1·0	13·0
Fraction 1	6·55	20	5	15	0·25	3·0
Fraction 2	6·70	1	0·5	0·5	0·1	0·8
Fraction 3	8·35	350	340	10	0·6	9·0
Lyophilized	8·95	350	330	20	1·1	9·0
Fraction 1	6·80	15	—	15	0·3	2·5
Fraction 2	7·00	2	0·1	2	0·08	0·8
Fraction 3	8·70	310	300	10	0·7	4·8
Dried (60 °C)	8·45	470	460	10	1·0	25
Fraction 1	6·70	15	10	5	0·2	1·0
Fraction 2	6·70	2	0·5	1·5	0·08	0·6
Fraction 3	7·00	440	430	10	0·6	22
Dried (100 °C)	8·10	840	825	15	1·2	28
Fraction 1	6·55	20	15	5	0·25	1·5
Fraction 2	6·50	7	4	3	0·1	0·6
Fraction 3	6·70	815	800	15	0·9	23

The data are related to 1 ml of pig slurry before fractionation. Fraction 1 contains compounds of high molecular weight and is eluted first. Fraction 2 is intermediate and fraction 3 contains compounds of low molecular weight. The coefficient of variation for the P analyses varied from 1 % at high concentrations to 10 % at very low concentrations and for the Cu and Ca analyses was about 5 %.

Table 3. *Calculation of the solubility product (see equation 1) of $CaHPO_4.2H_2O$ in pig slurry solution from the concentrations of calcium and phosphorus in the gel filtration fraction of low molecular weight compounds*

Description of slurry	$[Ca^{2+}]$ (mole/ 1×10^4)	[P] (mole/ 1×10^2)	pH	pK
Untreated	2·25	1·1	8.60	6.4
Lyophilized	1·2	0·95	8·95	6·8
Dried (60 °C, 12 h)	5·5	1·4	8·45	6·0
Dried (100 °C, 12 h)	5·7	2·6	8·10	5·7

Table 4. *Concentrations of some major and minor elements dissolved in pig slurry compared with values for total slurry. Characteristics of the slurry: pH = 8·6, D.M. = 6·3 %, dissolved $P_{inorganic}$ = 350 mg/l*

	Slurry solution (μg/ml)	Total slurry (μg/ml)
NH_4-N	4300	4400
Ca	10	1600
Mg	5	700
Fe	4·0	75
Al	1·5	35
Cu	1·0	6·0

of pure $CaHPO_4.2H_2O$ is given as $-\log K = 6{\cdot}56$ at 25 °C (Moreno, Brown & Osborn, 1960) which is in reasonable agreement with our data. The decrease in $-\log K$ with increasing drying temperature (Table 3) may be due to an increase in ionic strength, which was not taken into account, a change of mineral composition and/or the formation of complexes with low molecular weight compounds. In this respect formation of ion pairs and multi-ion complexes (Adams, 1971) should also be taken into consideration. In the case of Ca-soluble complexes as $CaHPO_4^0$, $CaHCO_3^+$ and $Ca(HCO_3)_2^0$ are most likely to be present under the conditions existing in pig slurry.

In addition to calcium, magnesium is also a major element in pig slurry. Together with ammonium and phosphate ions it can form a precipitate of $MgNH_4PO_4.6H_2O$ (struvite). The concentrations of magnesium and ammonium ions in pig slurry solution (Table 4) together with the pH and concentration of inorganic phosphate in untreated slurry (Table 2) and the calculated activity coefficients (Kielland, 1937), indicate a value of about -12 for log K. It is assumed that about a quarter of the dissolved magnesium is complexed by compounds of high molecular weight and the rest is present as free ion as was indicated for Ca in

Table 2. In the literature (Garrels & Christ, 1965) a value of $-13{\cdot}5$ was found for log K of struvite.

The concentrations of Fe and Al (Table 4) are found to be much higher than can be calculated by considering possible precipitates (e.g. strengite, variscite). Complex formation must thus be the main factor in determining the solubility of these cations in pig slurry.

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