PROTOCOL FOR BICARBONATE EXTRACTION OF INORGANIC PHOSPHATE FROM AGRICULTURAL SOILS

GITTE H. RUBÆK AND KRISTIAN KRISTENSEN

DCA REPORT NO. 102 · AUGUST 2017

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Preface

It has for many years been clear that the analysis for plant available phosphorus (bicarbonate extractable P also called Olsen P or in Danish "Pt or P-tallet") is not robust. This has been demonstrated and addressed in two previous reports: Rubæk and Sørensen (2011) and Rubæk (2015). In the latter, an updated but preliminary analytical protocol was presented, and it was recommended by the advisory board that several details in the protocol should be tested before a final update of the analytical protocol could be carried out.

To do this, The Danish Ministry of Environment and Food, Environmental Protection Agency commissioned and funded a project resulting in the present report.

The project has been supervised by an advisory board consisting of:
Søren Husted, Department of Plant and Environmental Sciences (PLEN), KU Science.
Leif Knudsen, SEGES
Esben Jensen, Agrolab
Ole Kristjansen, OKlab
Martin Frandsen, Eurofins Steins
Hans Estrup Andersen, Department of Bioscience, Aarhus University
Jørgen Eriksen, Department of Agroecology, Aarhus University
Karin Peters, Wibke Christel, Vinca Neergaard, The Environmental Protection Agency (board observers)
Charlotte Bruun Petersen, The Danish Agrifish Agency (board observer)

The project has been organised like this: Gitte Rubæk, Aarhus University drafted the plan on how to analyse the details in the analytical protocol specified in the previous report (Rubæk, 2015). The draft was sent to the advisory board for comments and suggestions. After revision, the plan was put out to tender among the laboratories presently carrying out Olsen P tests in Denmark. Agrolab won the tender and carried out the tests between September and end of November 2016. SEGES provided the soils for the tests. Gitte Rubæk and Kristian Kristensen, Aarhus University analysed the results of the six first tests presented here during December 2016. Based on this analysis, it was decided to do an additional lab test in early 2017 at Agrolab. The additional test was finalised on 7th of March 2017, after which a draft version of chapter 4 and 5 of this report was prepared by Gitte Rubæk and Kristian Kristensen and presented at a board meeting on April 24th. Based on the discussions and decisions at the board meeting, a draft of the board's recommendations (chapter 6) and an updated version of the analytical protocol (chapter 7) were prepared by Gitte Rubæk and send to the board for comments before being finalised. The final version also includes an analysis on the economic implications of the suggested changes for the farmers carried out by Leif Knudsen, SEGES (appendix 1).

We would like to thank the board for valuable input for this report, Maria Kreimeyer and Markus Rupprecht Agrolab for fruitful collaboration, competent and timely laboratory work and Anna Dorthe Østergaard for setting up the report.

Gitte H. Rubæk and Kristian Kristensen

July 2017

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1 Introduction

Analysis of soil P status with the bicarbonate extraction of P also "called Olsen P" or in Danish "P-tallet" or "Pt" has since 1987 formed the basis for fertiliser recommendations in Denmark. In response to the recognition of diffuse losses of P from agricultural land to surface waters as contributor to eutrophication of fresh waters a few decades ago, limitations for animal farms based on this measure of soil P status have been introduced in special cases in the legislation regulating animal husbandry in Denmark. In the new general legislation coming into force in 2017, ceilings on how much P can be applied for all types of P application are introduced. In this legislation, Pt has a central role since the ceiling can be increased if the average Pt on your farm area does not exceed specified limits.

This shift in the use of Pt from being an advisory tool for the farmer to being a regulatory measure based on which the size of the P ceiling is determined, necessitates even more than before a robust determination of the Pt, where the result does not depend on which laboratory carries out the analysis or when the soils are sent for analysis.

It has previously been demonstrated that Pt, which since 1994 has been carried out according the analytical protocol from Plantedirektoratet (1994), is not robust. The lack of robustness has been addressed in two previous reports: Rubæk and Sørensen (2011) and Rubæk (2015).

For the present report, the advisory board for the work presented in Rubæk (2015) was extended with representatives from OKlab and Eurofins Steins to ensure representation of all three soil laboratories offering P-tals tests on the Danish market. In Rubæk (2015), an updated, but preliminary analytical protocol, was presented based on discussions in the former version of the advisory board, but without practical laboratory testing. Thise advisory board strongly recommended that several of the details in the protocol should be tested in practise before a final update of the analytical protocol could be made. The aim of the present report is therefore:

- To present the results of practical tests of important details in the analytical protocol
- To summarise and communicate the recommendations and suggestions of the advisory board
- To formulate an updated and final analytical protocol based on the results of the tests and discussions in the advisory board to be used in routine soil analyses on Danish agricultural soils.

The key elements of the analytical protocol, which in Rubæk et al. (2015) were identified as in need for further clarification and specification in the analytical protocol, are given in table 1.1.

Table 1.1. Key elements in the protocol for bicarbonate extraction which need to be further specified. The comments from the laboratories on the draft protocol and the reasoning by the board for the previous work before giving their recommendation (reproduced from Rubæk, 2015).

Element in the existing protocols for bicarbonate extraction identified by the board as not sufficiently well described or in need of an update	**Comments from the laboratories**	**The board's rationale for its final recommendation**
Drying temperature of soil prior to analysis. The existing Danish method description stipulates drying at 50-60° C, while the ISO-standard (ISO 11263) stipulates 40° C. The board prefers a drying temperature of 40° C because of its reduced impact on the soil.	Some of the laboratories currently dry at 50-60° C and state that it will be costly and difficult for them to implement drying at 40° C, because the drying process will take longer which is not compatible with the drying capacity for the high number of soil samples processed daily.	The board decided to keep the drying temperature at 50-60° C for this preliminary version of the protocol. However, the aim is to reduce the temperature in the final version, after having documented and quantified the importance of this change in the protocol.
Amount of soil and dimensions of extraction containers. The dimensions of the container used for extraction should be specified in relation to the amount of soil (5 g) and extraction solution. The board is in favor of allowing less soil (1 g) for each extraction since this is common practice in research labs as it eases centrifugation and reduces the amount of chemicals needed.	One laboratory questions the decision to allow as little as 1 g of soil per analysis, as this might increase the variability of the result.	The board sticks to their first suggestion to allow smaller amounts of soil per analysis, but states in the protocol that variability might increase with smaller amounts of soil per analysis and that 5 g would be preferred for routine analyses.
Shaking method, type and speed of rotation. The board is in favor of end-over-end shaking because it is standard procedure in most soil labs and low speed because it minimizes disaggregation during shaking.	Some laboratories question the importance of this.	The board sticks to their first suggestion.
Temperature though out the extraction procedure. It is crucial that the temperature is kept at the specified level throughout the analysis until soil and solute have been separated. The board finds that it is reasonable to aim at the same temperature as used in the ISO-standard (ISO 11263, 1994) as that ensures direct compatibility with this standard and with proficiency test programs for this method.	Some laboratories state that keeping a lower temperature is challenging, especially in summer.	The board sticks to their first suggestion.
Time spent on extraction and handling of samples. Clear and narrow limits have to be specified for how much the extraction time can deviate and how much time can be allowed for handling samples after extraction.	The laboratories gave information on how fast a set of samples could be handled at present, and stated that the initially suggested time for handling of samples is unrealistic in their laboratory procedures.	The board has now specified the acceptable time limit for handling samples after extraction, which is less strict than our first suggestion but expected to be realistic in routine laboratories.

2 Tests of key details in the analytical protocol

In seven independent tests carried out at Agrolab, key details in the experimental conditions in the bicarbonate extractable P method are examined. An overview of the tests is given in table 2.1.

Table 2.1 Overview of the tests

Test number	Title	No. of variables	Soils	No. of replicate extractions	No. of test runs (repetition of the whole test in different weeks)
1	Drying temperature	2 (40 and 60 °C)	The ten fresh soils and four reference soils	4	2
2	Amount of soil	Two amounts (1 and 5 g)	The ten fresh soils and four reference soils	5	2
3	Speed of end over end shaking	2 speeds (20 and 30 rounds per minute)	The ten fresh soils and four reference soils	3	2
4	Temperature throughout the extraction procedure	4 temperatures (17, 20, 22 and 25°C)	The ten fresh soils and four reference soils	3	2
5	Time spent on handling the samples	4 time intervals (5, 20, 35, 125 minutes after end of extraction)	The 10 fresh soils and four reference soils	3	2
6	Method of separating the soil and solute after extraction	3 methods (manual filtration, robot filtration under pressure, centrifugation)	The ten fresh soils	3	2
7	Handling of solution between extraction and separation	Manual filtration, and centrifugation with or without resuspension prior to separation, centrifugation of entire suspension and filtration under pressure by robot without resuspension	Five of the fresh soils (B2, B4, B6, B8 and B10)	3	3

2.1 Materials and methods

2.1.1 Soils

Ten large fresh soils samples, approx. 5 kg of each, from agricultural fields in different regions of Denmark and four reference soils (Foulum Have 99, Liselund, Lolland 2000 and Troestrup 1995) also used in previous studies (Rubæk, 2015) were used. All soils were provided by SEGES. The soil samples were delivered to Agrolab in September 2016. The fresh soil samples were immediately sieved <4-6 mm mixed and divided into three portions while field moist. Two of these portions were stored field moist at 2°C until

further used. The third portion was dried at 40° C and sieved < 2 mm. Reference soil samples were already dried and sieved upon arrival at the laboratory. Sample codes and descriptions of the soils are given in table 2.1.1.

Table 2.1.1. Overview of the soils used in this test

Code in report	Type of soil	Status on arrival to the lab	Crop (previous crop)	Rt	Humus (%)	Clay (%)	Silt (%)	Fine sand (%)	Coarse sand (%)
A01	Reference soil "Lolland 2000"	Air dry, sieved < 2mm		7.3	1.9	16.2	15.7	40.6	26.0
A02	Reference soil Foulum 99 Græs"	Air dry, sieved < 2mm		6.5	5.6	7.6	12.1	42.0	32.8
A03	Reference soil "Jens K Mark"	Air dry, sieved < 2mm		6.2	5.0	4.1	4.3	41.6	46.3
A04	Reference soil "Liselund"	Air dry, sieved < 2mm		6.6	2.7	12.2	13.3	41.9	29.6
B01	Køge, Zealand	Field moist	Winter wheat (winter wheat)	6.3	2	10	10.7	46.4	30.7
B02	Ringsted, Zealand	Field moist	Spring barley (winter barley)	-	-	-	-	-	-
B03	Ringsted, Zealand	Field moist	Winter rape (spring wheat)	6.7	2.3	11.4	11.2	46.9	28.3
B04	Vemb, Jutland	Field moist	Potatoes (spring barley)	5.7	1.8	3.4	1.6	28.3	64.9
B05	Vemb, Jutland	Field moist	Potatoes (spring barley)	6	1.9	3	1.5	27.1	66.5
B06	Vemb, Jutland	Field moist	Potatoes (spring barley)	6.1	2	2.3	0.8	20.6	74.2
B07	Bredebro, Jutland	Field moist	Spring barley (spring barley)	5.9	5.6	4.8	2.1	47.4	40.1
B08	Vojens, Jutland	Field moist	Spring barley (spring barley)	6.2	7.1	6.3	3.6	29.5	53.6
B09	Brovst, Jutland	Field moist		6.3	2.4	4.2	2.8	75.0	15.7
B10	ØsterVrå, Jylland	Field moist		6.3	-	-	-	-	-

2.1.2 Experimental setup

The seven key details in the analytical protocol for bicarbonate extraction of P (table 2.1) were tested in seven independent tests as summarised in table 2.1. All tests were carried out at AGROLAB, Agrar und Umwelt GmbH, Sarstedt in Germany. In each test, other factors than the one being tested was kept constant. Apart from the factor varied in each test, the analysis was carried out according to Rubæk, (2015) with the following specifications: (1) Soil was dried at 40° C, (2) 5 g of soil was used, (3) temperature was kept constant at 20^0 C, (4) shaking was end over end at a speed of 20 rounds per minute

and (5) separation was carried out by simple filtration. Handling time between end of extraction and separation was maximum 15 minutes. Test 1 to 6 were repeated twice (test a and b) with at least one week's time lapse between each test run. Test 7 was repeated three times with at least one week's time lapse between each test run.

TEST 1. Drying temperature

For each of the independent test run (a and b), 10 field moist samples stored at 2° C and four reference soils were used. From each of these, four individual subsamples of approximately 100 g were sampled and dried at 60° C until they did not lose weight any more. Another four ca. 100 g subsamples were dried at 40°C until they did not lose weight any more. The four reference soils were also included in this experiment. Bicarbonate extraction of P was made in triplicate from each of the dried subsamples. All P measurements in the extracts were carried out in the same analytical batch.

TEST 2. Amount of soil

The result and the uncertainty (when performing the test on only 1.0 g (i.e. 0.95 - 1.04 g) without correcting the final result for the actual weight gram of soil) were compared to the traditional 5.0 (i.e. 4.95 – 5.04 g) grams of soil. The solution volume to volume of container was kept constantly at 1:50. The ten fresh soils dried at 40° C and four reference soils were used. The extractions were made with five replications. Measurements were carried out in the same analytical batch. There were two independent test runs (a and b).

TEST 3. Speed of end over end shaking

Two shaking speeds considered to be realistic for implementation in routine soil laboratories and possible to carry out at Agrolab at present (20 and 30 rounds per minute) for end over end shaking were compared. Other shaking methods were not investigated. This test was carried out on the dried batches of the ten fresh soils dried at 40° C and the four reference soils in three replicates. There were two independent test runs (a and b).

TEST 4. Temperature throughout the extraction procedure

The ten fresh soils dried at 40° C and four reference soils. Four extraction temperatures of 17, 20, 22, and 25° C +/- 1.0° C were compared. This test was done with three extraction replications. Temperature was measured in extraction solution prior to initiation of extraction and checked again right after extraction and after separation. There were two independent test runs (a and b).

TEST 5. Time spent on handling the samples after shaking

The effect of handling time after end of shaking and before separation was tested on the 10 fresh soils dried at 40° C and four reference soils. The handling times were 5, 20, 35, and 125 minutes after extraction.

This test was carried out with triplicate extractions. There were two independent test runs (a and b).

TEST 6. Method of separating the soil and solution after extraction

Classical filtration using funnels and filter paper, fast filtration under pressure (robot filtration) and centrifugation at 1800 g for 5 minutes at 20°C was compared in this test. The test was carried out with triplicate extractions on the 10 fresh soils. There were two independent test runs (a and b).

TEST 7. How to treat suspension prior to different separation methods

This test was carried out as a follow-up on test 6 to clarify details around separation further. Six combinations (A to F) of separation methods and how to handle suspension after extraction but before separation were included:

A. The upper layer of solution, after the soil has been allowed to settle at the bottom for 15 minutes, was sampled with a syringe. The removed solution/suspension was transferred to a centrifuge tube and centrifuged as described for test 6 prior to P analysis
B. The upper layer of solution, after the soil has been allowed to settle at the bottom for 15 minutes, was sampled with a syringe. The removed solution/suspension was filtered by simple filtration using funnels and filter paper before P analysis
C. The upper layer of the suspension was sampled automatically (robot) and filtered under pressure before P analysis
D. After extraction, the suspension is left for 15 minutes to settle before the extraction containers are gently shaken to stir up the settled material immediately before a subsample is retrieved for centrifugation as in A.
E. After extraction, the suspension is left for 15 minutes to settle before the extraction containers are gently shaken to stir up the settled material immediately before the entire mixture is transferred to for simple filtration as in B.
F. After extraction, the whole container is transferred to centrifugation as in test 6. Here only 1 g of soil and 50 ml of extraction were used in order to be able to transfer the extraction container directly into the centrifuge

Five soils were used. In each test run, there were triplicate extractions with each soil and method. There were three independent test runs (a, b and c).

2.1.3 Statistical analyses

The results from the seven tests were analysed with four different models:

1. A linear mixed model that included the effect of "Soil", "Treatment" (which was specific for each test, see second row of table 1), "Soil x Treatment", "Run" and "Soil x Run" as fixed effects. The effect of "Soil.Treatment within Run" and "Subsample" were included as random effect. For Test 1, where each subsample was first subdivided in 8 subsamples the random effect of this subdivision were included in the model.
2. In order to test if the temperature after Shaking and Filtration had any effect on the results, the above model was extended by including the effect of "temperature after shaking" and the "temperature after filtration". This analysis was only relevant for test 4.
3. In order to test if the variation between subsamples was dependent on one of the factors, a model with the fixed effects as in model 1 and the random effect of "Soil.Treatment within Run" were used to analyse the logarithm of the standard deviation between subsamples for test 1 (as the four larger subsamples of each soil was dried individually at each temperature prior to replicating the analysis on each subsample). Log-transformed standard deviations were used in order stabilize the variance – and thus better fulfil the assumption for the analyses.
4. In order to test the factorial structure in test 7, an additional analysis was performed on treatment A, B, D, and E where those 4 treatments were considered as a two by two factorial.

2.1.4 Analysis results obtained with the reference conditions across all test runs in all seven tests

The reference treatments from all tests were combined into one data set including also the actual date of the analyses. This dataset was used to check how robust the analysis has been in this study. This model included the effect Soil and Date and the interaction between those as fixed effect. This model was also used to analyse the standard error between subsamples.

2.2 Results and discussion

In all seven tests, there was as expected a significant effect of soil, merely demonstrating that the Pt in the test soils differ from each other in soil P status. The treatments were significant in test 3 (shaking speed rmp), test 5 (time after shaking before separation), test 6 (separation method) and test 7 (suspension and separation method) when using model 1. In test 4, (extraction temperature) the effect of Initial Extraction Temperature became significant when the results were adjusted for temperatures measured after shaking and after filtration when using model 2 (table 2.2.1).

Table 2.2.1 Summary of variance components and significant effects for different test-datasets and three different analyses. 1) ANOVA for testing the effect of Soil. Treatment and some interactions; 2) The same ANOVA, but now including the effects measured temperatures after shaking and after filtration (only for test 4); 3) ANOVA for testing if the standard Error between Subsamples depended on Soil, Treatment, Run and some interactions; 4) For testing the effects the effect of four of the treatments in a factorial structure.

Effect*	**Test 1**	**Test 2**	**Test 3**	**Test 4**	**Test 5**	**Test 6**	**Test 7**
Treatment	Drying temperature	Amount of soil extracted	Sha-king speed	Tempera-ture during extraction	Time after shaking	Separa-tion method	Handling prior to separation
1) ANOVA mean							
Random effects							
Soil.Treatmt.Run	*0.0309*	*0.0030*	*0.0465*	*0.0655*	*0.0084*	*0.0933*	*0.0033*
Subdivision	*0.0552*						
Subsample	*0.0616*	*0.0640*	*0.3653*	*0.0501*	*0.0712*	*0.0777*	*0.0141-0.485*
Fixed effects							
Soil	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Treatment	0.7732	0.8833	0.0040	0.1349	<.0001	<.0001	<.0001
Soil* Treatment	0.0112	0.2999	0.9472	0.9999	<.0001	0.0140	<.0001
Run	0.0798	0.8188	0.0040	0.0334	<.0001	0.0002	<.0001
Soil*Run	0.0005	0.6381	0.8387	0.6483	0.0003	0.2897	0.1316
2) ANOVA+Regr							
Random effects							
Soil.Treatmt.Run				*0.0471*			
Subsample				*0.0514*			
Fixed effects							
Soil				<.0001			
Treatment				0.0070			
Soil* Treatment				0.9983			
Run				0.0008			
Soil*Run				0.4277			
Shaking Temp				0.0057			
Filtration Temp				0.3598			
3) ANOVA ln(SE)							
Random effects							
Soil.Treatmt.Run	*0.0000*						
Subsample	0.6344	*0.3309*	*0.4576*	*0.3508*	*0.4677*	*0.5203*	*0.4702*
Fixed effects							
Soil	<.0001	0.0022	0.0121	<.0001	0.0002	0.0051	<.0001
Treatment	0.4797	0.0002	0.7075	0.0001	0.1164	0.4276	0.0030
Soil*Treatment	0.7061	0.9537	0.3627	0.2540	0.5570	0.7021	0.0717
Run	<.0001	0.4671	0.8165	0.8336	0.0027	0.1465	0.3750
Soil*Run	0.0032	0.4735	0.2150	0.2529	0.0103	0.1144	0.5831
4) ANOVA							
Random effects							
Soil.Treatmt.Run							0.0059
Subsample							.0098-.608
Fixed effects							
Separation							0.0168
Resuspension							0.0632
Sep*Resusp							0.6424
Soil							<.0001
Sep*Soil							0.0396
Resusp*Soil							0.1548
Run							0.0004
Soil*Run							0.3523

*)Analyses number/name are shown in bold. Values in italic are variance components. Other values are P-values.

In several cases, Soil interacted with Treatment and/or Run. Only weight, Extraction temperature and handling prior to separation (test 2, 4, and 7 tested with model 3) had a significant effect on standard deviation between subsamples. In all test-datasets, soil had a significant effect on the standard deviation (model 3, table 2.2.1). This was also the case for the standard error on all the reference observations from all seven TESTs as shown in fig. 2.2.1. Here standard errors and Pt for all 14 soils are shown. There is a tendency that soils with high Pt also have higher standard errors, but the relation is not straightforward (fig. 2.2.1). When comparing standard errors for the 14 soils pairwise, the standard error was significantly different at the 5 percent level for the soils in 31 out of 91 possible pairwise comparisons.

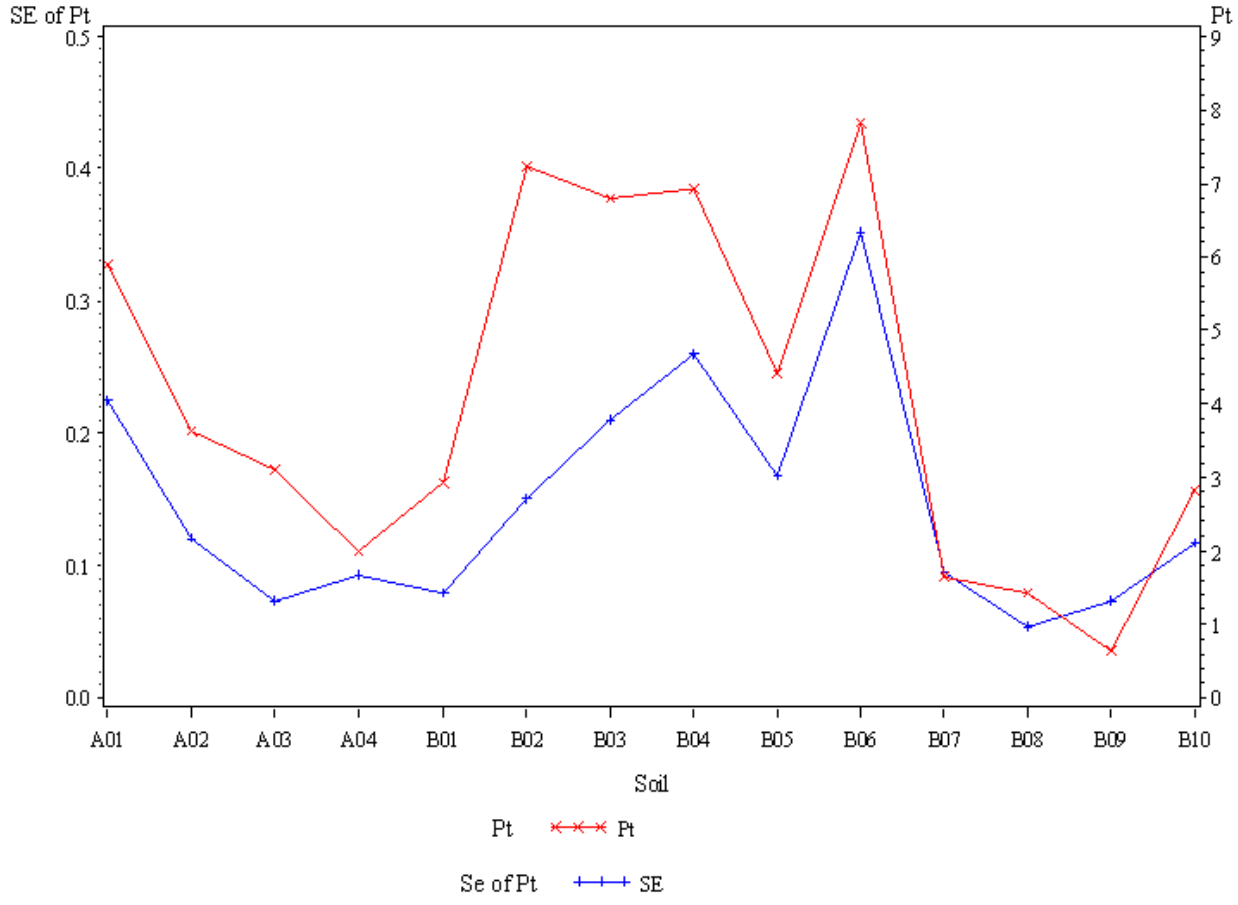


Figure 2.2.1. Pt and standard error (SE) for the 14 soils analysed with the reference conditions in TEST 1 to 7 in this study.

For more details on the effect of different treatment factors, see the tables in the appendix 2. Each test is further discussed in the following sections.

2.2.1 Drying temperature for soil samples prior to analysis

Drying the soil at 60° C gave on average similar results as drying at 40° C; however, there was a significant interaction between drying temperature and soil, where for some soils drying at 40° C resulted in higher Pt values than drying at 60° C, while for other soils the opposite was observed (figure 2.2.1.1, table 2.2.1 and table A1). Subdivision of moist samples in each test round introduced additional variation, but did not affect the mean value of the measurement significantly (table 2.2.1). Other conditions during the drying process other than the actual temperature (time, aeration, air humidity etc.) are most probably also influential on the final Pt measurement, but temperature was in focus of this investigation because it was judged by the board to be of major importance.

To minimize the differences that may occur on some soils if dried at different temperatures and to stay in accordance with the ISO standard for soil pre-treatment (ISO 11464), we therefore recommend to set the maximum allowed drying temperature for soil samples prior to Pt-analysis to maximum 40° C.

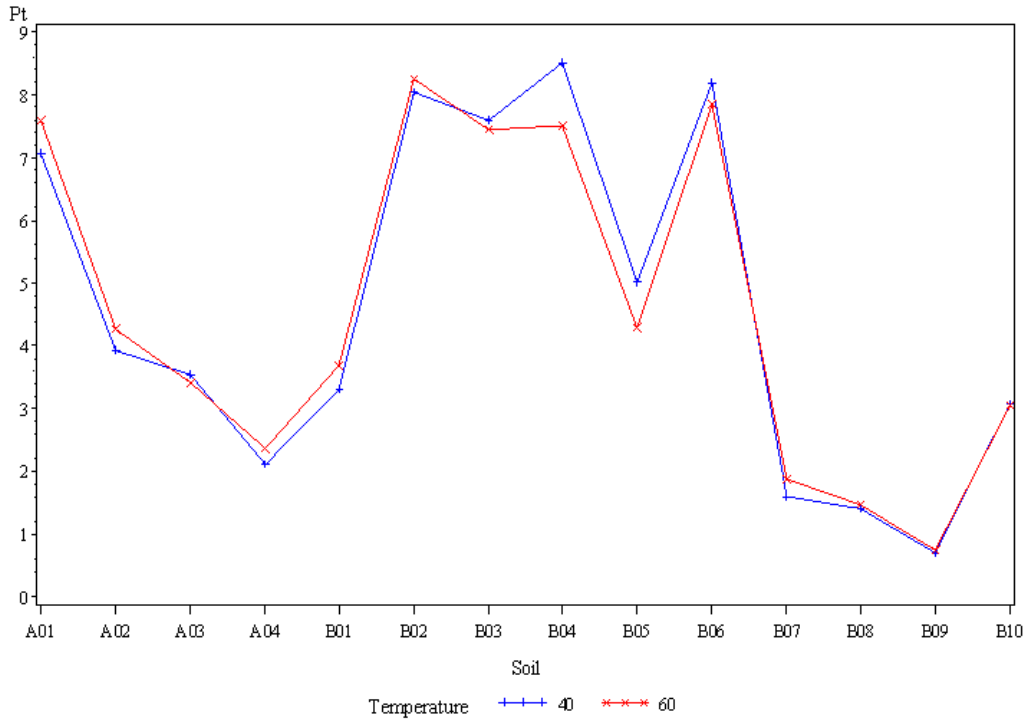


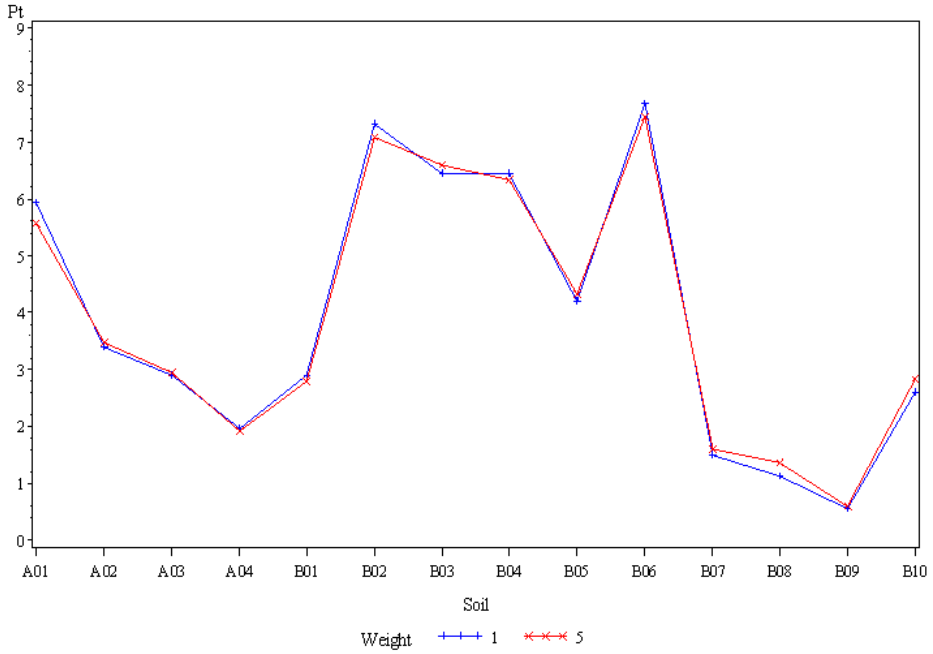
Figure 2.2.1.1. Effect of drying temperature on the amount of P extracted from four reference soils and 10 fresh soils- Unit of Pt is mg P/100 g soil. Soil codes correspond to the description given in table 2.1.1.

2.2.2 Amount of soil for extraction

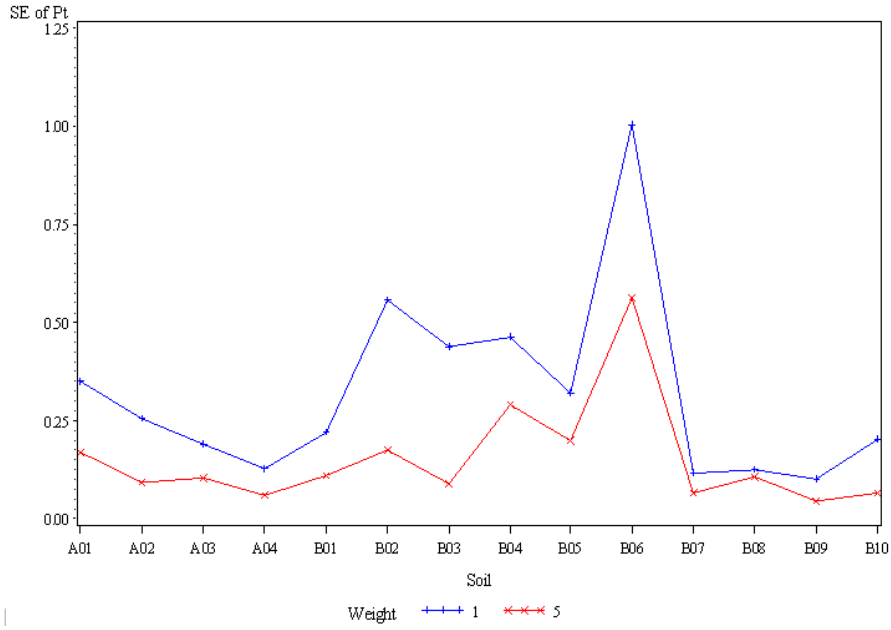
The amount of soil used for the extraction did not influence the result of the Pt-determination (fig. 2.2.2.1a, table 2.2.1 and table A2), but weighing only one g of soil instead of 5 grams did increase the standard

error associated to the determination (fig. 5.2.2.1b, table 2.2.1 and table A2).

For routine purposes, we therefore recommend using 5 grams of soil for analysis, but analysing 1 g is an option e.g. when the size of the soil sample is limited.



a)



b)

Fig. 2.2.2.1 ***a:*** *The effect of the amount of soils used for extraction on extracted P on the 14 soils. Unit of Pt is mg P/100 g soil. Soil codes correspond to the description given in table 2.1.1.* ***b:*** *The standard errors of mean (back transformed) for extractions of 1 and 5 grams of soil on the 14 soils.*

2.2.3 Speed of shaking

Shaking speed for end over end shaking of 30 rounds per minute resulted in significantly higher Pt values (average of 4.15 mg P/100 g soil) than 20 rounds per minute (average of 3.91 mg P/100 g soil) (table 2.2.1, fig 2.2.3.1 and table A3.). There is therefore no doubt that there is a need to standardize the shaking intensity. A certain level of mixing or shaking is needed to ensure good and even contact between soil and extractant, but vigorous shaking should be avoided due to the risk of abrasion (McKeague and Cline, 1963; Hans Christian Bruun Hansen, personal communication) i.e. hard soil particles such as quartz during extraction damage less resistant particles.

We therefore recommend that shaking speed for end over end shaking is fixed at as low as possible speed, while still ensuring good mixing which in practice would be 20 rounds per minute.

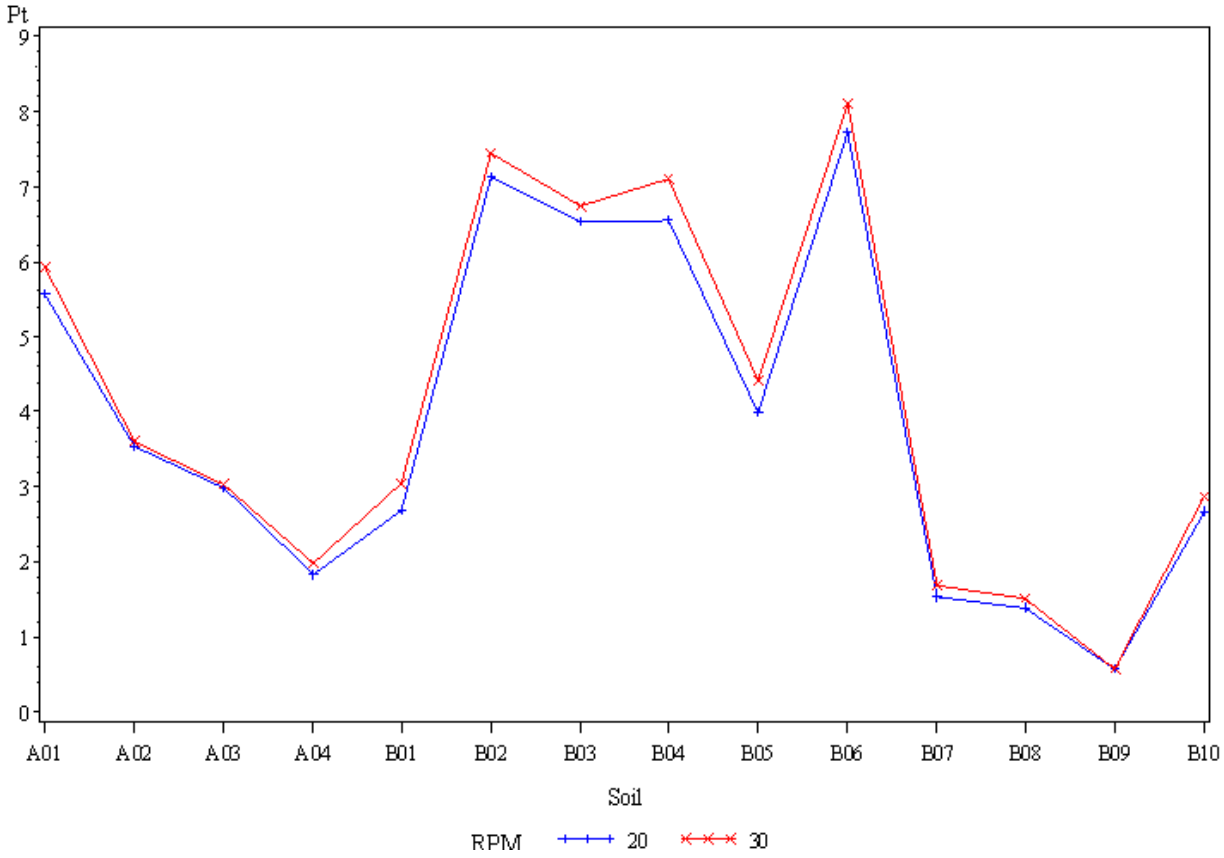


Fig. 2.2.3.1. The effect of shaking speed (rounds per minute, RPM) on the amount of extracted P on 14 soils. Unit of Pt is mg P/100 g soil. Soil codes correspond to the description given in table 2.1.1.

2.2.4 Temperature throughout the entire extraction procedure

The effect of temperature during extraction was insignificant in the tested interval between 17° C and 25° C, when only the initial/planned temperature was taken into account (Model 1) (table 2.2.1 and fig. 2.2.4.1 and table A4.) The planned extraction temperature became significant (more P was extracted with increasing planned temperature) when the temperature measured after shaking and after filtration was taken into account (Model 2) (fig. 2.2.4.2 and table A5). With this model, the effect of the temperature after shaking also became significant with decreasing Pt values with increasing temperature after shaking. The decrease was estimated to be 0.156 Pt per °C. The standard error on the estimate was 0.056 Pt per °C. The effect of measured temperature after filtration was positive, but far from being significant (P=0.3598). It is surprising that an increase in temperature after shaking leads to decreases in the measured Pt values; we had expected the opposite. It is clear that the temperature had not remained constant at the temperatures planned in this experiment. This is further illustrated in the plots of the measured temperature after shaking against initial (planned) temperature in Appendix 3. The largest increases in temperatures were seen for the extraction planned at 17° C, and the largest decreases in temperature were seen for the planned extractions at 25° C. There was furthermore a large variation in the resulting temperature for all planned temperatures and a significant effect of test run (table 2.2.1 and figures in appendix 3).

It was possible to track the exact position of each extraction container in each shaking. Therefore, we also tested inclusion of this information in an additional statistical test in order to determine whether changes in temperature and test results were related to the exact position of each container during extraction. This could be the case if the shaking apparatus it-self had a temperature gradient depending on closeness to the motor. This test revealed that position had no significant effect on the test result or on the temperature changes experienced during extraction (data not shown).

The lack of control of temperature throughout the extraction procedure has compromised the initial intention of this experiment, namely testing the importance of different, but constant temperatures during the entire extraction procedure. It is therefore not possible to make firm conclusions regarding temperature based on this experiment. But temperature is clearly an important factor for this extraction method and we suggest that temperature throughout the entire extraction procedure is kept constant at 20 ° C $\pm$1 ° C in the final protocol, which is in line with the ISO standard (ISO 11263) for the bicarbonate extraction and also suggested in the previous report (Rubæk et al., 2015).

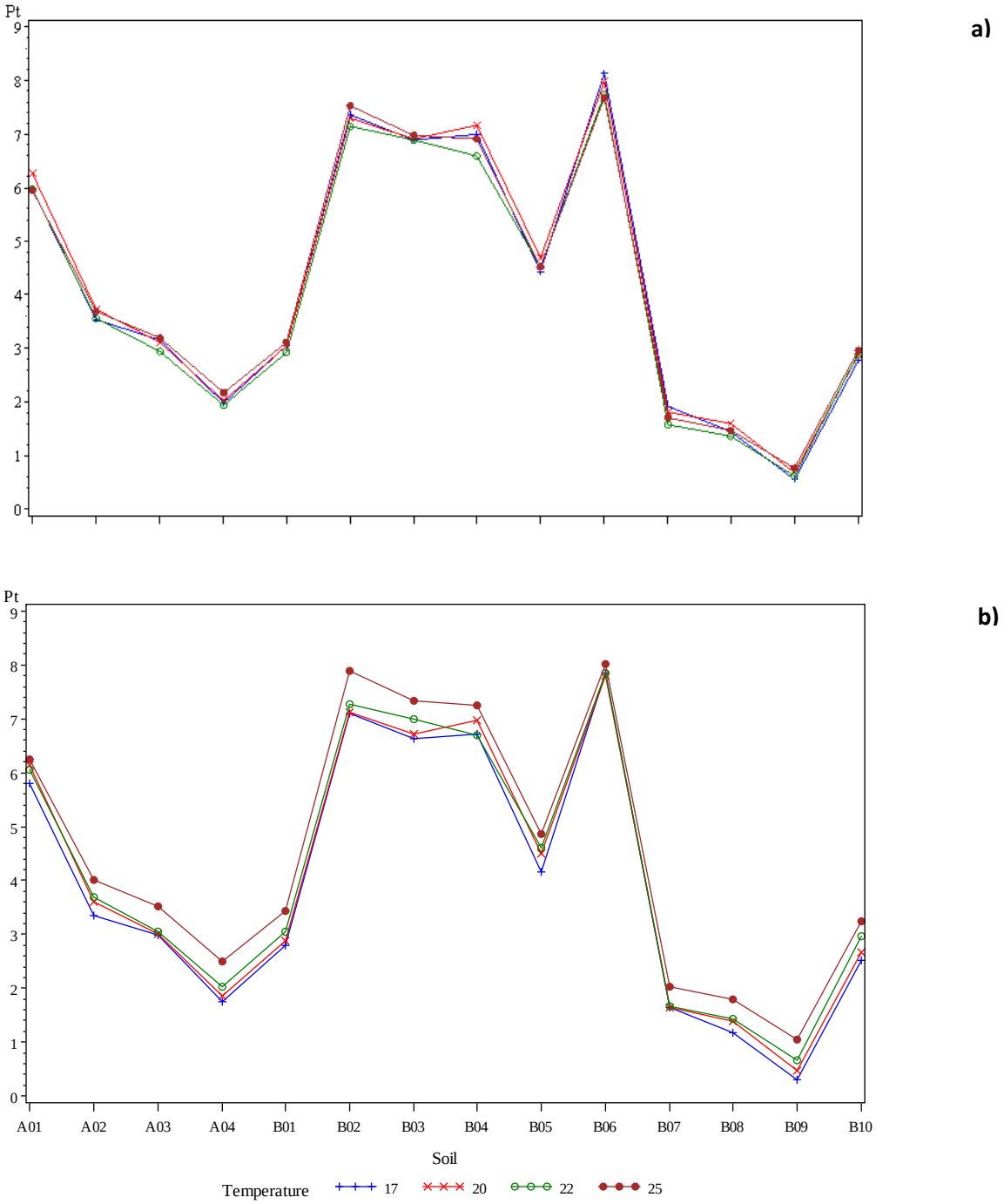


Fig. 2.2.4.1. ***a)*** *The importance of Temperature on extraction of P from 14 soils* *without adjusting for temperature after shaking and filtration* ***b)*** *The importance of Temperature on extraction of P from 14 soils when adjusting for temperature after shaking and filtration. Unit of Pt is mg P/100 g soil. Soil codes correspond to the description given in table 2.1.1.*

2.2.5 Handling time after shaking

We carried out the present test with traditional separation of extract and soil, i.e. after standing the designated time; the soil was re-suspended again when it was transferred to the filter. Time after shaking had significant influence on the average Pt measurements made (average increased from 3.64 to 4.52 when handling time increased from 5 to 125 minutes (table 2.2.1 and fig 2.2.5.1 and table A7). In addition, the effect of time interacted significantly with soil type resulting in an even more pronounced effect on some soils, e.g. soil B04, where Pt was 5.77 with 5 minutes handling time compared to 7.62 with a handling time of 125 minutes and B06 were Pt was 6.56 after 5 minutes and 8.88 after 125 minutes handling time.

We recommend that handling time after shaking before separation should be limited to maximum 5 minutes prior to separation especially when separation is carried out by traditional filtration with resuspension in connection with transfer to the filter.

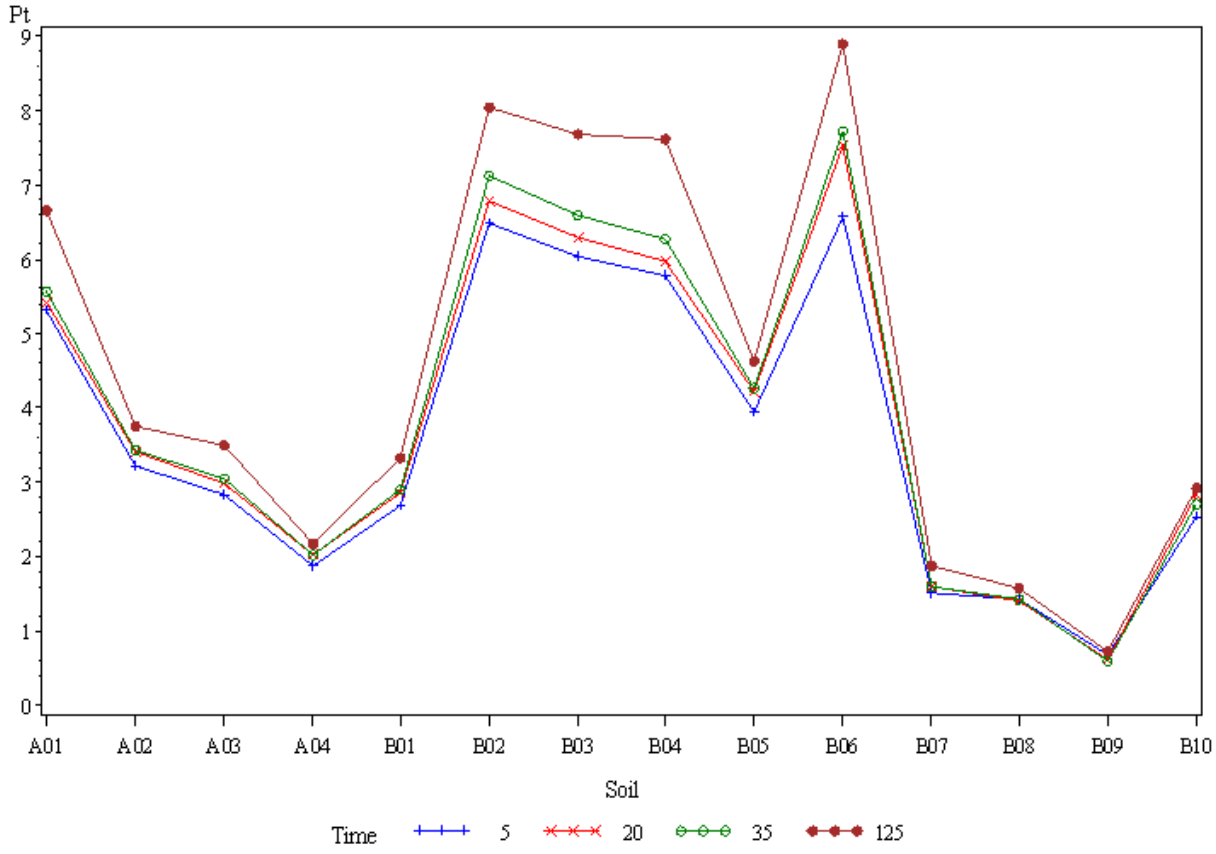


Fig 2.2.5.1. The importance of time after extraction before separation by simple filtration for extractable P. Unit of Pt is mg P/100 g soil. Soil codes correspond to the description given in table 2.1.1.

2.2.6 Method of separation soil and liquid after extraction

Method of separation had significant effect on the measured Pt values. Centrifugation resulted in the highest values, and robot filtration under pressure gave the lowest values (table 2.2.1 and fig 2.2.6.1 and table A8.). Furthermore, soil and separation method interacted significantly. This was a surprise to us, until we carefully scrutinized every single step in the procedures for each separation method as they were carried out in this test, and we realized that for centrifugation the whole mixture of soil and liquid were decanted into centrifuge beakers and thereby re-suspended before centrifugation, while when centrifugation is carried out e.g. at AU-Agro, the whole tube used for extraction is centrifuged under temperature control immediately after end of extraction time, and thereafter never re-suspended. For the traditional filtration using filter paper and funnels in this experiments, soils were also re-suspended prior to filtration, but handling time/re-suspension was probably shorter than for centrifugation since it is a routine procedure at Agrolab, whereas centrifugation is not. For the robot filtration, extraction tubes were left to settle and never shaken again before a sample for filtration was taken out in the upper layers of liquid.

We therefore conclude that a separation method seems to have important implications for the resulting Pt value. Furthermore, the way the sample is handled after end of shaking and until separation may turn out as important factors too, and this should be tested subsequently in a test where both separation method and resuspension/no resuspension in connection with the separation are varied systematically and where time between shaking and separation is fixed (TEST 7). In the final protocol, the handling procedures after end of shaking should be described in more detail.

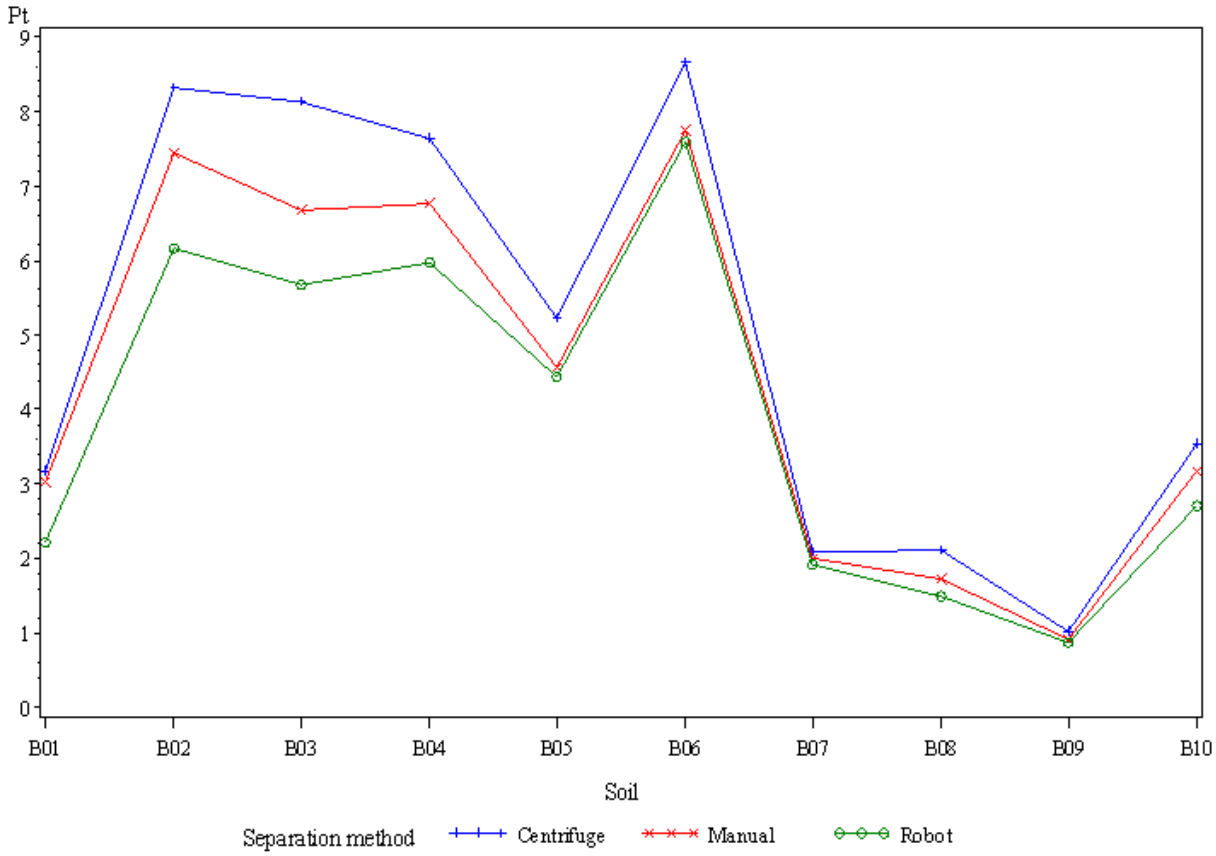


Fig. 2.2.6.1. The importance of the separation method for how much P is extracted estimated on 14 soils. Unit of Pt is mg P/100 g soil. Soil codes correspond to the description given in table 2.1.1.

2.2.7 Test of combinations of resuspension and separation

In this test, the robot filtration under pressure gave far lower estimates of Pt than all other tested combinations of resuspension and separation on soils B02, B04, and B06 and these three soils had relatively high levels of extractable P. On soil B10 and B8, with lower levels of extractable P, there were differences among the tested combinations of resuspension and separation method as well as significant interactions between treatment and soil (fig 2.2.7.1a and table A9). In the statistical analysis, this was expressed as a significant interaction between soil and separation procedure (table 2.2.1). When traditional filtering is used, there is a risk that filters are clogging up, whereby the filtration process is slowed down allowing further P extraction during filtration resulting overestimation of the Pt values. This problem is minimized by filtration under pressure and by centrifugation. It is likely that this error is more pronounced on soil with high P content and fine texture and this may explain the observations made in this study.

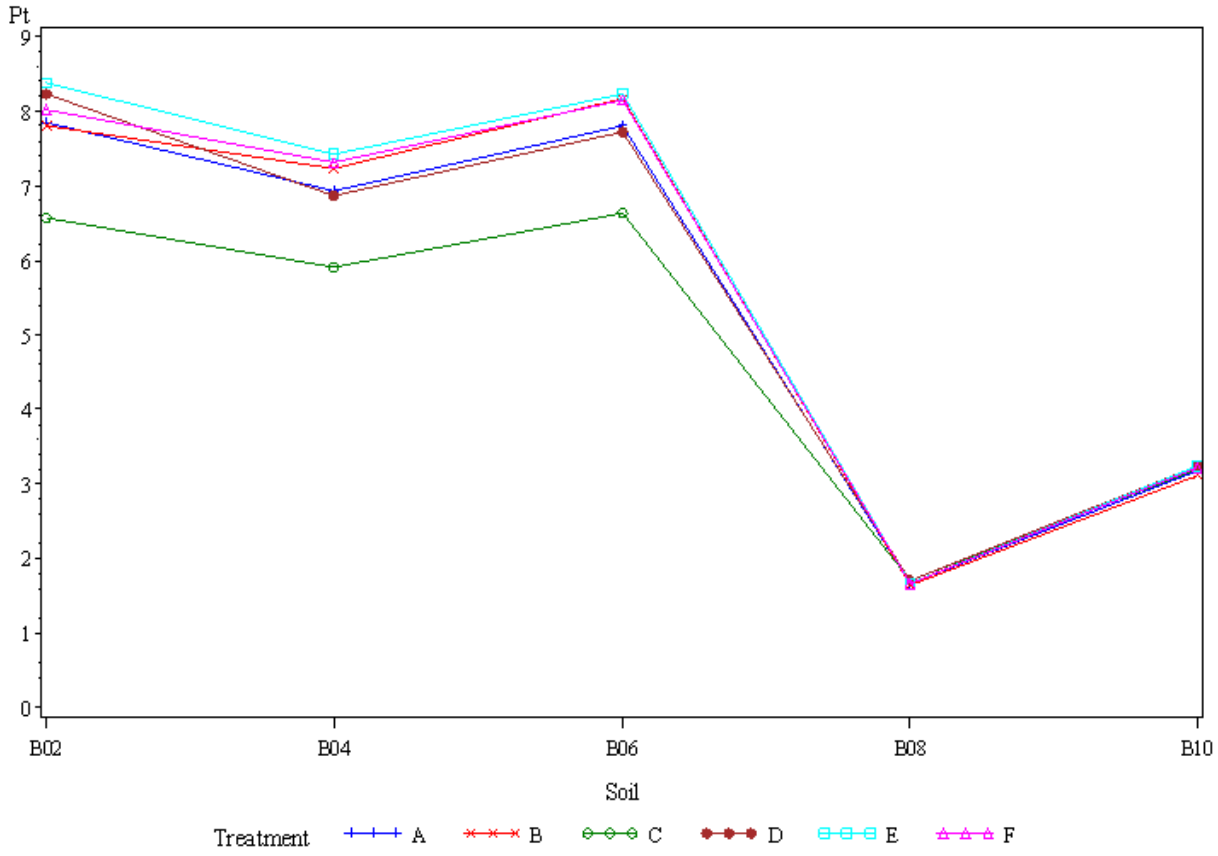


Fig. 2.2.7.1. Test of six procedures for separation and handling of suspension prior to separation on five different soils. All extraction containers are left standing and settling for 15 minutes. A represents sampling of liquid from container for centrifugation without resuspension B sampling of liquid like A, but for simple centrifugation. C represents robot filtration under pressure after automated sampling of liquid without resuspension. D is sampling for centrifugation after resuspension, D is simple filtering after resuspension and F is centrifugation of the entire soil liquid mixture without resuspension.

The treatments A, B, D, and F were also tested in a two factorial design with separation (by filtration or centrifugation) and resuspension (with and without) as the two factors. In this analysis, centrifugation yielded a significant (P=0.0168) lower Pt value than filtration and the difference depended on the soil (table 2.2.1, fig 2.2.7.2), that is the effect was more pronounced on soil B04 and B06 than on the other soils. The effect of resuspension was not significant in this experiment, but it should be noted that handling time after shaking was only 15 minutes, which may have been too short for this effect to turn out significantly with this experimental set-up.

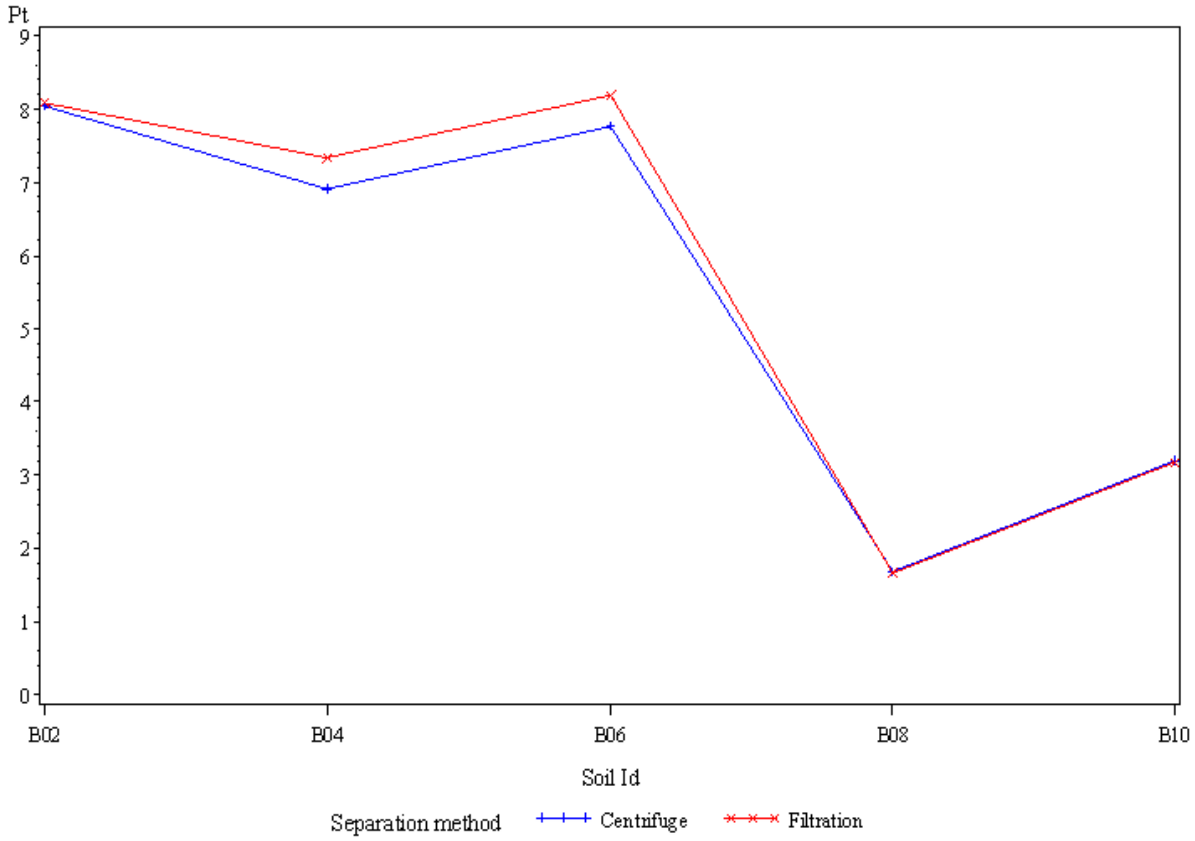


Figure 2.2.7.2. Interaction between Separation methods and Soils. Centrifugation and filtration were compared with a two factorial model.

The estimated standard errors varied significantly among treatments A to F (fig. 2.2.7.3), but not as much as they did among soil types (fig. 2.2.7.3, fig. 2.2.7.4 and table A10).

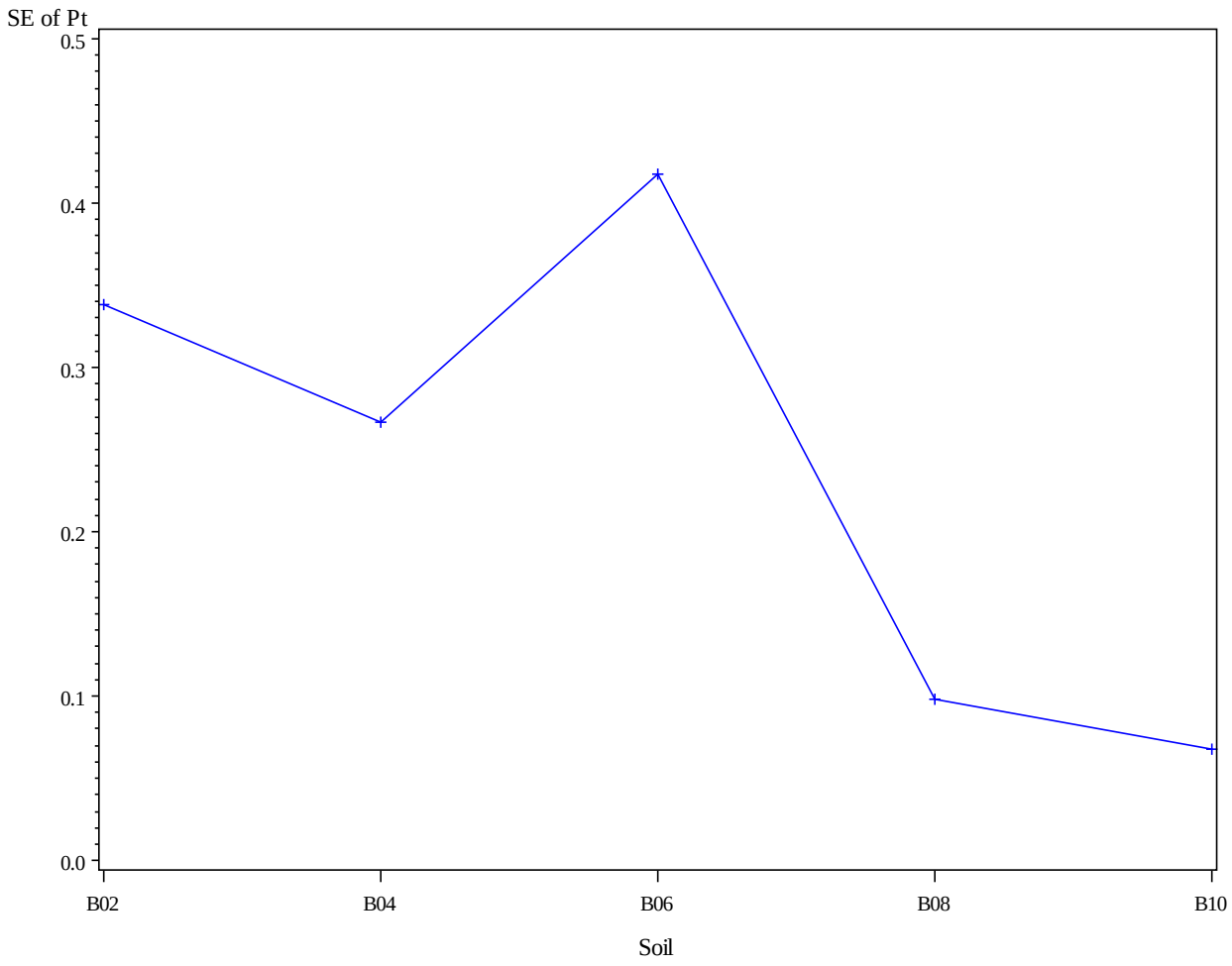


Figure 2.2.7.3. Estimates on standard errors for the different soils in test 7.

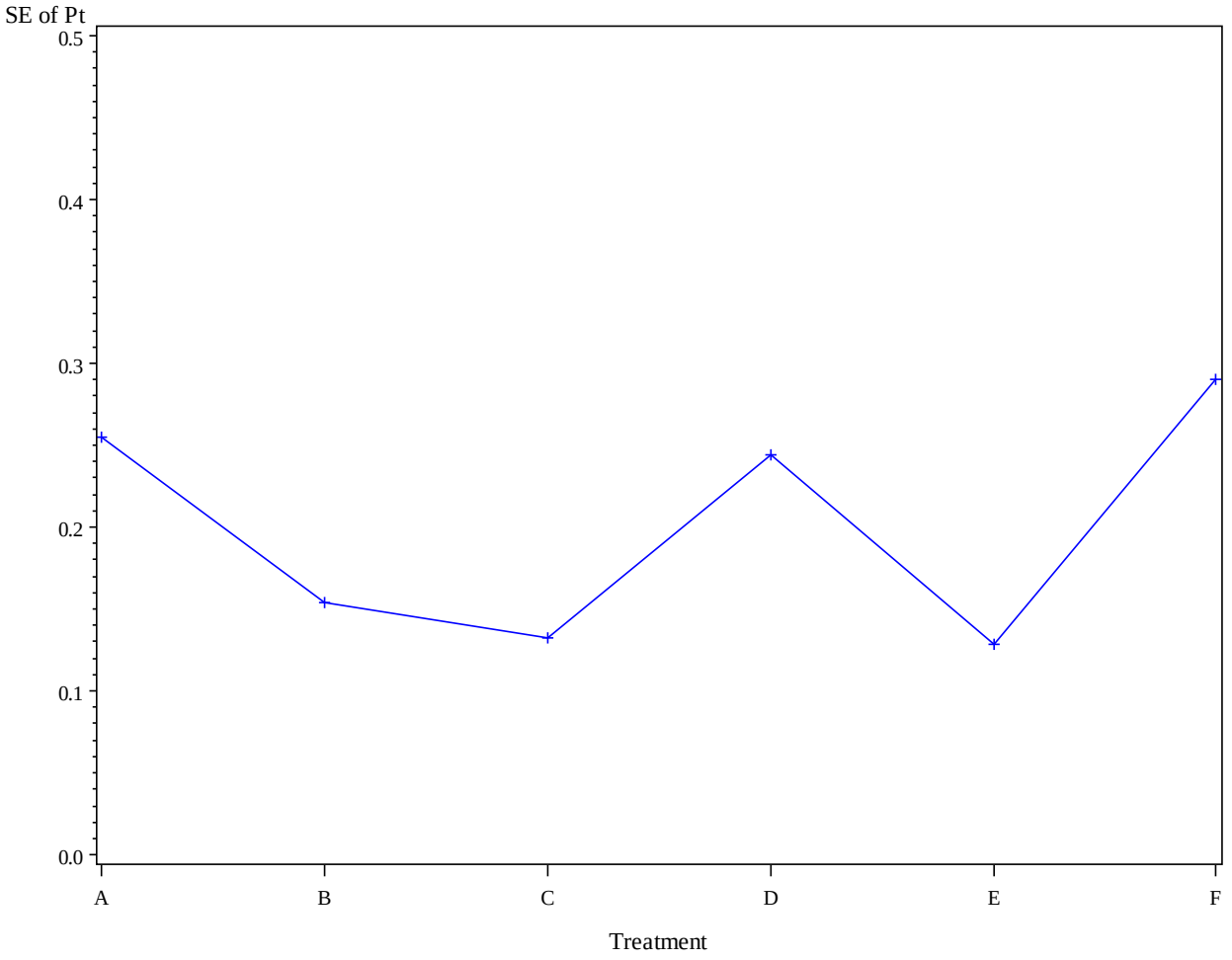


Figure 2.2.7.4. Estimates of standard error for the different combinations of separation and resuspension in test 7.

We conclude that separation method does influence the test result and the associated error on some soils, but the effect is not pronounced.

2.2.8 Variations in test results due to date of analysis

The test results varied significantly with time for analysis (fig 2.2.8.1.) The variation was more pronounced for soils with relatively high values for Pt. This indicates that the main underlying problem causing the analysis not to be robust was present and unsolved for the reference settings during this study.

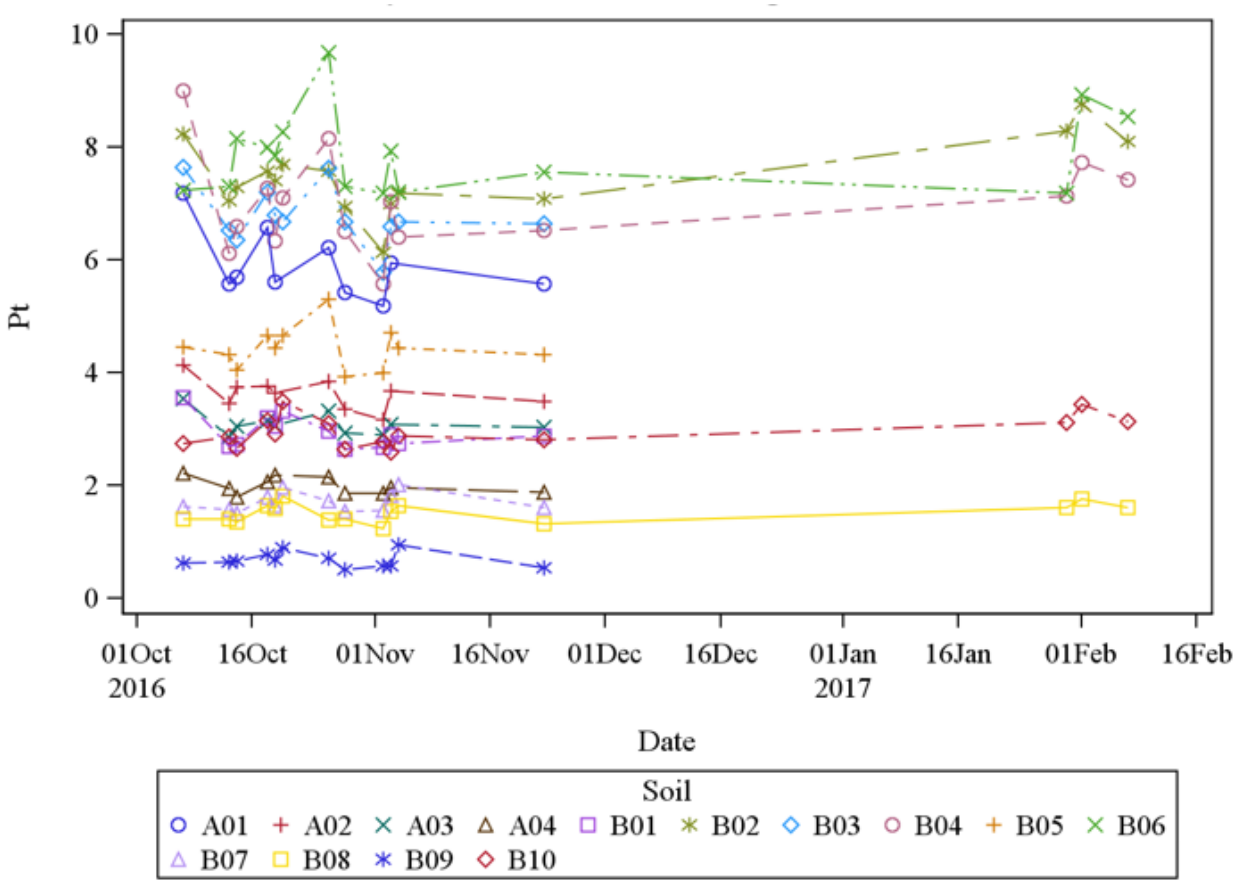


Figure 2.2.8.1. Estimates of P obtained for each soil in the seven individual tests for the reference conditions plotted against date of analysis. The reference conditions are according to Rubæk 2015 with these additional specifications relating to the tested variables here: Soil drying temperature was 40° C, 5 g of soil was used for analysis, temperature was 20° C ±1 during extraction, shaking speed was 20 rounds per minute, separation was carried out as filtration with resuspension and maximum 15 minutes handling time was allowed between end of extraction and separation.

2.2.9 The authors' recommendations and conclusions

Based on the results obtained here and the previous work on the validity of the Olsen soil P test, the authors of this report recommend the analytical protocol to be updated on the following issues:

1. Drying temperature has an effect on the measured Pt value and should be standardised, and high drying temperatures should be avoided. We recommend 40° C as this is in accordance with ISO standards.

2. Amount of soil should be 5 g in accordance with ISO standards, but reducing to 1 g (with down-scaling the amount of extractant) is an option in non- routine studies with low amounts of soils available. The precision of the weight is 5.00 in the ISO standard. In the present study, a precision of 5.0 ±0.1 was used and we consider this to be acceptable.
3. Temperature for extraction should be kept at 20° C ±1° C (in accordance with the ISO standard) and kept constant throughout the entire extraction and separation procedure.
4. Shaking speed should be specified in the analytical protocol and kept as low as possible. In practise, 20 rounds per minute is recommended.
5. Time after extraction until separation should be kept at a minimum. The ISO standard states immediately after end of extraction and we agree with this and suggest specifying this further to "maximum 5 minutes after end of extraction" even though we are aware that this is not applicable in routine labs at present.
6. Resuspension of the soil prior to separation should be avoided when samples are allowed to stand for more than 5 minutes after end of extraction and prior to separation.
7. It can be argued that centrifugation or fast filtration under pressure would give more valid results than traditional filtration due to the fact that traditional filters clog and filtration is slow allowing for additional extraction. However, the effect was small in this study and we therefore recommend that separation method is optional allowing both filtration and centrifugation.

The abovementioned updates and clarifications in the analytical protocol are expected to improve the robustness of the analysis, but they alone may not result in a sufficiently robust method (i.e. without significant variations in results due to laboratory or time of analysis). To obtain robustness, further and formalised quality control measures are needed. This could encompass sharing common reference soils among the laboratories and using these reference soils in each test round at each laboratory either for correction as suggested in Rubæk and Kristensen (2015) or for setting common and sufficiently narrow quality control ranges for the test result of these soils for acceptance or rejection of the test run. Also abandoning the long term tradition of single determinations and setting limits for accepted deviation multiple determinations could most probably increase the validity/robustness of the obtained results significantly.

3 Summary of the advisory board's recommendations

The outcome of the seven tests described in chapter 5 and the authors' preliminary conclusions and recommendations were presented and discussed at a whole day advisory board on April 24th 2017. The board's recommendations were outlined in the minutes from the meeting and summarized in this chapter. A draft of this summary has been circulated for further comments and corrections among the board members and this final version of this chapter has been approved by the advisory board.

3.1 Recommendations for the final protocol for P-number analysis:

3.1.1 Drying temperature for soils prior to analysis

The board recommended that the drying temperature must be fixed in the protocol and that the present situation where some commercial laboratories dry soils at 40° C others at 60 ° C is inexpedient. Drying at low temperature is preferable according to the scientists' conclusion in chapter 2 and 40° C is in accordance with the ISO standards (ISO 11464 and ISO 11263). However, lower drying temperatures require longer time and therefore larger oven capacity for drying, which is costly to implement at laboratories presently drying at 60° C. The board could not agree upon which drying temperature to recommend. The final decision was therefore taken by the Environmental Protection Agency. Their decision is to set the drying temperature to 40° C in accordance with the ISO standards.

3.1.2 How much soil to use in each extraction

The advisory board recommend that routine soil analyses should be carried out on 5 grams of soil, but with the possibility to downscale the extraction to 1 g of soil in special cases where only small amounts of soil is available.

3.1.3 Precision of the weight of soil

The preliminary protocol (and the standard ISO 11263) prescribes weighing with a precision 5.00 grams, but during the board meeting, it became clear that few if any of the laboratories worked with this precision in practise. The general opinion of the board was that high precision is redundant taking other and potentially more influential sources of uncertainty into consideration. The laboratories states that reaching this precision will increase the costs of the analysis and worsen the working conditions for the lab technicians significantly. It also became clear that the strategy for weighing in soils was different at the labs. Some weigh 5.0 ±0.1 and continue without recording and correcting for the actual amount of soil used. Others weigh between 4 and 6 grams of soil with two decimals precision, record the actual weight and correct for actual amount of soil in the result of the test. The board found that weighing in between 4 and 6 grams of soil without adjusting the amount of extractant accordingly is a significant deviation from the protocol and that further documentation would be needed before this can be accepted. A

compromise regarding precision, which is acceptable for all three laboratories, is to recommend weighing 5.0 ±0.1 without recording the actual weight or as an alternative to weigh 5.0 ±0.3 g and adjusting the final result for the actual amount of soil used can be accepted. Both procedures should be described in the final protocol.

3.1.4 Speed of shaking during extraction

The board recommends that the speed of shaking should be fixed and identical at all labs (this is not the case presently) to eliminate the small differences in results which may arise from different shaking intensities. The board finds that the actual speed, which is chosen, is mainly of academic relevance. The preliminary protocol specifies shaking speed at 20 rounds per minute, the former protocol (Plantedirektoratet 1994) specifies 40 rounds per minute, while the ISO standard does not specify speed. The board first decided to change shaking speed to 40 rounds per minute, to stay in accordance with the former Danish protocol, and decided that expert advice should be sought at Copenhagen University. The advice obtained here (Hans Christian Bruun Hansen, personal communication) and supported by Aarhus University was to choose the lowest shaking speed, which is possible in practise, the board in the written process after the meeting decided to recommend the shaking speed fixed at 20 rounds per minute.

3.1.5 Extraction temperature

Even though problems occurred in this test in relation to keeping temperature constant during extraction, the board is convinced about the importance of constant temperature and recommends that the temperature for extraction should be 20 ± 1° C which is in agreement with the preliminary protocol and the ISO standard, ISO 11263, but lower than the former protocol, (Plantedirektoratet, 1994). The importance of keeping the temperature constant during the entire extraction and until soil and liquid is separated should be stated very clearly in the final protocol.

3.1.6 Time between end of extraction prior and separation of soil and liquid

Based on the results presented in chapter 5 and on the practical difficulties with very narrow time intervals but in spite of the scientists recommendations, the board recommends that the allowed time interval should be extended from 15 minutes to 30 minutes. Furthermore it should be specified in the protocol that resuspension of the soil before separation is not allowed.

3.1.7 Separation method

The board recommends that both separation by filtration and centrifugation should be allowed, since the difference caused by separation method alone was minor and the costs for the laboratories to change would be large.

3.2 The board's additional recommendations

3.2.1 Quality control

The board also wishes to raise the attention on the importance of continued commitment from the ministries and the stakeholders involved in this board for future development and recommendation regarding appropriate soil analyses and a quality insurance, i.e. proficiency test programs for inter-laboratory comparison by means of reference soils as presently carried out in DK by SEGES. A more elaborate recommendation on this was given in the previous report (Rubæk, 2015).

3.2.2 Handling of soils with low density (organic matter rich soils)

The board wishes to raise the attention on this highly relevant but often neglected issue of low density organic matter rich soils. This has not been addressed directly in this or the two previous reports on soil and P analyses, and the problem is relevant for other soil analyses too. Some of the tricky issues regarding such soils are:

- The protocol for soil analyses in Denmark (Plantedirektoratet, 1994) specifies how differences in soil density can be handled by converting measurements made "by soil weight" into "by volume". A simple additional analysis is required for this, which is most often not considered. This is a flaw when comparing soils with different bulk densities.
- It is unclear whether is the lab's responsibility or the person who submits the sample to decide whether a density analysis is required.
- In practise, it can be difficult to weigh in 5 grams of a low density soil for P analysis. It is simply too voluminous for the extraction containers.
- The Pt method is typically not recommended for organic soils, but the transition between mineral soils and organic soils is gradual. Which alternative method to use, and when are they to prefer?

4 Protocol for bicarbonate extractable P (Pt or P-number)

4.1 Principle

Phosphorus is extracted from soil with a sodium bicarbonate solution at pH 8.5 for exactly 30 minutes at 20° C ± 1° C, after which soil and solution are immediately separated. In the clear filtrate/supernatant, the concentration of the blue phosphomolybdate complex is measured by spectrophotometry after adding sulphuric acid, ascorbic acid, and ammonium molybdate reagent to the extract.

This method extracts only a modest proportion of soil total P and can therefore be very sensitive to small deviations in extraction time and temperature and intensity of shaking. It is therefore very important that the temperature should is kept at 20° C ± 1° C from initiation of the extraction until soil and solute are separated. The bicarbonate extractant can produce coloured soil extracts, which may precipitate upon acidification of the extract during the colorimetric determination of P. These problems are handled by addition of polyacrylamide to the extracting solution as described by Banderis et al. (1976).

4.2 Apparatus

- Rotating shaking apparatus "end-over-end", shaking intensity 20 ± 2 rounds per minute.
- Scale for measuring 1-5 grams with two decimal places.
- Analytical scale with 5 decimal places.
- Acid-washed bottles and lids and glassware (or similar of material suitable for soil and analysis of phosphorus i.e. materials which do not adsorb phosphate).
- Spectrophotometer or similar for determination of light absorbance at wavelength 880 nm.

4.3 Reagents

All reagents shall be analytically graded and water should be purified (resistivity at 25° C of maximum 18.2 MΩ cm.

4.3.1 4M sodium hydroxide solution

Dissolve 160.0 g sodium hydroxide (NaOH) pellets in 700 ml water. Cool and dilute to 1000 ml with water. Store the solution in an inert and hermetically sealed bottle.

4.3.2 Polyacrylamide solution

Polyacrylamide (Granular powder MW over 5.000.000, BDH Laboratory supplies prod. no. 297883N or similar) approx. 0.05% water solution. Dissolve 0.10 g polyacrylamide in 200 ml water. Note that it takes several hours to dissolve the polyacrylamide.

4.3.3 Extracting solution

Dissolve 210 g of sodium hydrogen carbonate ($NaHCO_3$) in 4500 ml water. Add 25 ml of the polyacrylamide solution (4.3.2). Adjust the pH to 8.50 ± 0.02 with the 4.0 M sodium hydroxide solution (4.3.1) while stirring. Add water to 5000 ml volume. The solution should be prepared and sealed within 10-15 minutes. If hermetically sealed, it can be kept for weeks. However, pH should be controlled daily and a new solution should be prepared if pH deviates from 8.50 ± 0.04.

4.3.4 4M Sulphuric acid

In a fume hood, pour *ca.* 350 ml of water into >1000 ml container, add 110.0 ml concentrated (95-97%) sulphuric acid (H_2SO_4) while stirring, cool to room temperature, and add up to 500 ml volume.

4.3.5 0.1M Sulphuric acid

Dilute 4.0 M sulphuric acid (4.3.4) 40 times with water, by adding 25 ml 4.0 M sulphuric acid to approx. 900 ml water and fill up to 1000 ml volume with water.

4.3.6 Ammonium molybdate potassium antimonyl tartrate solution (Sulfomolybdic reagent)

a. Dissolve 13.0 g ammonium heptamolybdate-tetrahydrate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) in 100 ml water
b. Dissolve 0.35 g potassium antimonyl tartrate ($K(SbO)C_4H_4O_6 \cdot 0.5\ H_2O$) in 100 ml water
c. In a fume hood, add approx. 120.0 ml concentrated sulphuric acid (95-97%) into approx. 170 ml water while stirring, and cool to room temperature. Mix solution "a" and "b" into the diluted sulphuric acid and fill up to 500 ml with water. Store the reagent cool (2-5° C) and protect against sunlight.

4.3.7 Ascorbic acid solution

Dissolve 5.00 g ascorbic acid ($C_6H_8O_6$) in water and dilute to 100 ml volume.

4.3.8 Stock solution

Stock solution of 200 mg P/l. Dissolve 1.7573 ±0,0002 g dried potassium dihydrogen phosphate (KH_2PO_4) in 2000 ml volume of 0.1 M H_2SO_4 (4.3.5).

4.3.9 Standard solutions

Prepare standard solutions with concentrations of PO_4-P ranging from 0 to 8 ppm as suggested in table A4 by appropriate dilution of the stock solution with the extracting solution (4.3.3).

Table A2: Concentrations of P in standard curve solutions and the amount of stock solution (4.3.8) to transfer to 100 volumes to obtain these concentrations.

PO4-P concentration (mg/l)	Amount of stock solution (3.8) (µl) to dilute with extracting solution (3.3) to 100 ml volume
0	0
0.1	50
0.2	100
0.5	250
1.0	500
3.0	1500
5.0	2500
8.0	4000

4.4 Procedure

4.4.1 Extraction

Weigh 5.0 ±0.1 g soil (dried at max. 40°C until constant weight, sieved <2.0 mm and mixed) into 250 ml flask or container. Alternatively, weigh 5 ±0.30 g dried soil (with two decimals precision) and correct the final result for the actual amount of soil used in the extraction). In special cases with little soil available for the extraction, the amount of soil can be down-scaled to 1.00 g soil, but soil weight to container volume ratio has to be 1:50 and soil to solution ratio has to be 1:20 (w:v). The temperature of the mixture of soil and extractant (4.3.3) has to be 20° C ± 1° C from the start of the extraction to end of separation. Close flasks immediately and mount them on the shaker for exactly 30 minutes at 20 ± 1° C. Separation of soil and solute by either centrifugation of samples at minimum 1800 g for 5 minutes at 20 ±1° C or by filtration. Handling time between end of shaking and end of separation must not exceed 30 minutes. Suspensions which are not immediately separated must not be re-suspended prior to separation. When separation is carried out by filtration, the first milliliters of filtrate should be discarded.

Prepare blanks following the same procedure, but excluding soil.

4.4.2 Measurement

Transfer 1 ml of extract quantitatively to a beaker large enough to handle foaming and bubbles upon acidification (25 ml Erlenmeyer flasks work well. Handling in racks makes work easier). Add 9 ml of water and 125 µl 4.0 M H_2SO_4 (4.3.4). Swing flask and leave for CO_2 evolvement and foaming to cease. Then add 400 µl ascorbic acid solution (4.3.7) and swing. Add 400 µl of the sulfomolybdic solution (4.3.6) and swing.

A standard curve is produced by transferring 1 ml of each standard solution and adding water, acid, and reagents the same way as to the samples.

Flasks are left for 10-15 minutes at room temperature for color development to complete. The blue color is typically stable for up to 24 hours. The color intensity of the samples and standards are measured on a spectrophotometer at 880 nm. Use the zero standard for setting zero. A path length of 1 cm of the cuvette is appropriate for most measurements, but at concentrations of less than 0.25 mg/l the path length should be 4 cm or more. Make sure that bubbles of CO_2 do not obstruct the measurement.

If blanks do not produce zero absorbance or very close to (less than 0.004), the analysis should be repeated. A thorough check for contamination of reagents, bottles, and glassware can be necessary.

If the soil extract is highly colored, it should be tested if this color absorbs light at 880 nm and if it does corrections for this absorbance will be necessary.

Automated procedures for measurements are accepted, as long as they rely on the principle described above of measuring the intensity of the blue color developed after addition of the above-mentioned reagents.

4.5 Calculations

Carry out a linear regression of measured absorbance of standard solutions against their known concentrations of P according to this equation:

$Abs_{st} = \alpha * C_{Pst}$

Where:

Abs_{st} is the measured absorbance for each standard solution,

C_{PSt} is the known P concentration in each standard solution,

and α is the constant derived from the regression line crossing the origin.

P concentration in the soil extracts can then be calculated as:

$P_{cons\_extract} = (Abs_{sample} - Abs_{blank})/\alpha$

The amount of bicarbonate-extractable P in mg P kg^{-1} dry soil can then be calculated as:

P extracted = $P_{cons\_extract}*20$

Detection area is 2 to 160 mg Olsen P kg^{-1} soil.

If the result is requested as the Danish Ptal, the result should be divided by 10 and the unit is then mg P extracted per 100 g of soil.

4.6 Repeatability

Reference soils should be included in every analytical run. The standard deviation of independent measurements on the reference soils measured at different times in the same laboratory with the same equipment should be less than 10% of the measured value or less than 2 mg P kg^{-1} soil (0.2 P-tal units).

4.7 Test report

A test report shall contain the following:

a. A reference to this method description
b. All information necessary for complete identification of the sample
c. Results of the determination in whole numbers in milligram per kilogram calculated on the basis of dried sieved soil (dried at max. 40°C)
d. Any details of operations not specified in this method description as well as any other factors, which may have affected the results.

4.8 Comments

This method description is an update of the former Danish method description (Plantedirektoratet, 1994) and of the preliminary protocol published in Rubæk (2015). It corresponds in major aspects to the ISO 11263:1994 and to the original method description by Olsen et al. (1954).

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Appendix 1 – Relation between the price of the Olsen-P analyses and value for the farmers

6/7- 2017, Leif Knudsen, SEGES

In Denmark, the Olsen-P soil testing method is used to estimate the amount of plant available phosphorous in soils. The farmers use the results directly as an input to planning the P fertilizer application of P to the single fields. Determination the Olsen-P in soils is furthermore used as a mandatory parameter in different aspects of the Danish legislation for application of animal manure on farmland. For farmers using the derogation from the nitrate directives limiting the application of N to 170 kg N/hectare in animal manure, it is mandatory to tests the soil for Olsen-P extractable P every 4 years. Additionally, there is a possibility for farmers to increase the limits for phosphorus application from fertilizers by documenting the average Olsen-P level in the soils being below 4.0 mg P/100 g soil.

The analysis of Olsen-P is included in the standard package of analyses for soil testing together with analyses of pH (Rt), potassium (Kt), and magnesium (Mgt). Most farmers are testing their soils for these standard parameters every 4 to 7 years. Sampling density is between 0.2 to 1 sample per hectare. In total approximately 100.000 soil samples are analyzed in Denmark every year. The cost for a package with the four standard soil analyses is about 40-50 kr. per sample. The cost for the analysis alone should be compared with a total cost per sample at 120-200 kr. for sampling, mapping, and interpretation.
If the costs for the Olsen-P analyses will increase significantly because of optimizing the robustness of this analysis, the consequence might be that farmers deselect Olsen P when they do soil testing in general, and only prioritize to have the Olsen-P measured on fields, which are suspected to have P deficiency. This might reduce the total number of P-analyses from 100.000 to a level of 10-20.000. It is, however, not possible to estimate an exact number for how much the cost for Olsen-P analyses should increase before the farmers will give this analysis a low priority when ordering standard soil analyses. However, it is assumed that the Olsen-P analyses is valuable for the farmers and a decrease in the annual number of Olsen P analyses will only be expected if the cost will increase significantly (maybe more than 25 pct.). If a moderate price increase is a result of an improvement of the test result with a higher robustness and a lower systematic error of the analysis, it will be regarded as an advantage for the farmers. On the other hand, the current problems with the known systematic differences between laboratories and between the time of analysis at each laboratory could also be a reason for excluding Olsen-P from the standard soil test package if these problems remain unsolved.

Farmers who needs Olsen-P analyses to get the right to use the derogation rules according to the nitrate directive or to prove low Olsen-P status to increase their P-quotas, will under all circumstanced need Olsen-P-analyses even if the price increase significantly.

Based on the response from the laboratories involved in this current project, it is estimated that the suggested changes to the Olsen-P measuring protocol will in some cases increase the cost for the analysis. However, as the changes to the protocol are very limited, the increase is considered to be modest, and it is therefore not expected that it will affect the total number of annually soils samples being tested for Olsen-P extractable P in Denmark.

Appendix 2 – Test results from statistical analyses

Averages (least square means) and standard errors (SE) given in the tables below are obtained from the models shown in table 2.2.1.

Table A.1 Mean Pt for each soil and drying temperature in test 1

Soil	Drying temperature		
	40° C	60° C	Average
A01	7.07	7.59	7.33
A02	3.92	4.26	4.09
A03	3.53	3.42	3.48
A04	2.12	2.37	2.24
B01	3.31	3.69	3.50
B02	8.04	8.26	8.15
B03	7.60	7.44	7.52
B04	8.50	7.51	8.01
B05	5.01	4.28	4.64
B06	8.18	7.85	8.01
B07	1.60	1.87	1.74
B08	1.41	1.48	1.45
B09	0.70	0.75	0.72
B10	3.07	3.05	3.06
Average	4.57	4.56	

Table A.2 Mean Pt and SE for each soil and amount of soil in test 2

	Mean Pt			SE of Pt	
Soil	Amount of soul				
	1 g	5 g	Average	1 g	5 g
A01	5.92	5.58	5.75	0.35	0.17
A02	3.39	3.48	3.43	0.25	0.09
A03	2.90	2.95	2.92	0.19	0.10
A04	1.97	1.92	1.94	0.13	0.06
B01	2.91	2.79	2.85	0.22	0.11
B02	7.30	7.07	7.19	0.56	0.17
B03	6.44	6.59	6.51	0.44	0.09
B04	6.44	6.33	6.38	0.46	0.29
B05	4.21	4.32	4.27	0.32	0.20
B06	7.68	7.43	7.56	1.00	0.56
B07	1.49	1.60	1.55	0.12	0.06
B08	1.14	1.37	1.25	0.12	0.11
B09	0.57	0.60	0.58	0.10	0.05
B10	2.59	2.84	2.72	0.20	0.07
Average	3.93	3.92		0.26	0.12

Table A3 Mean Pt for each soil and speed of shaking in test 3

Soil	Speed of shaking		
	20 rpm	30 rpm	Average
A01	5.56	5.93	5.75
A02	3.55	3.60	3.57
A03	3.00	3.02	3.01
A04	1.84	1.98	1.91
B01	2.69	3.05	2.87
B02	7.13	7.45	7.29
B03	6.53	6.74	6.63
B04	6.56	7.11	6.83
B05	3.99	4.41	4.20
B06	7.73	8.11	7.92
B07	1.53	1.70	1.61
B08	1.39	1.51	1.45
B09	0.59	0.59	0.59
B10	2.66	2.89	2.77
Average	3.91	4.15	

Table A4 Mean Pt for each soil and temperature in test 4 without adjusting for temperatures after shaking and filtration

Soil	Temperature during extraction				
	17° C	20° C	22° C	25° C	Average
A01	5.96	6.26	5.96	5.96	6.03
A02	3.54	3.72	3.57	3.57	3.63
A03	3.16	3.12	2.95	2.95	3.11
A04	1.99	2.02	1.94	1.94	2.03
B01	3.05	3.06	2.93	2.93	3.04
B02	7.35	7.28	7.14	7.14	7.32
B03	6.88	6.90	6.89	6.89	6.91
B04	6.99	7.16	6.58	6.58	6.91
B05	4.43	4.69	4.53	4.53	4.54
B06	8.12	7.97	7.75	7.75	7.88
B07	1.91	1.82	1.57	1.57	1.76
B08	1.45	1.59	1.37	1.37	1.47
B09	0.57	0.68	0.63	0.63	0.66
B10	2.78	2.88	2.90	2.90	2.88
Average	4.16	4.23	4.05	4.19	

Table A5 Mean Pt for each soil and temperature in test 4 after adjusting for temperatures after shaking and filtration

Soil	Temperature during extraction				
	17° C	20° C	22° C	25° C	Average
A01	5.79	6.13	6.06	6.25	6.06
A02	3.34	3.61	3.69	4.01	3.66
A03	2.99	3.01	3.05	3.52	3.14
A04	1.75	1.86	2.03	2.51	2.04
B01	2.79	2.88	3.04	3.44	3.04
B02	7.11	7.12	7.27	7.88	7.35
B03	6.63	6.71	6.99	7.34	6.92
B04	6.71	6.98	6.68	7.25	6.90
B05	4.15	4.51	4.62	4.87	4.53
B06	7.84	7.80	7.84	8.01	7.87
B07	1.64	1.64	1.66	2.04	1.74
B08	1.17	1.39	1.43	1.79	1.45
B09	0.31	0.47	0.67	1.06	0.63
B10	2.52	2.66	2.96	3.24	2.85
Average	3.91	4.05	4.14	4.51	

Table A6 Mean SE of Pt for each soil and temperature in test 4

Soil	Temperature during extraction				
	17° C	20° C	22° C	25° C	Average
A01	0.37	0.26	0.30	0.22	0.28
A02	0.16	0.14	0.16	0.05	0.12
A03	0.08	0.01	0.06	0.02	0.03
A04	0.05	0.05	0.19	0.04	0.07
B01	0.07	0.08	0.17	0.06	0.09
B02	0.15	0.10	0.24	0.12	0.14
B03	0.15	0.19	0.37	0.12	0.19
B04	0.24	0.27	0.23	0.10	0.20
B05	0.18	0.18	0.23	0.05	0.14
B06	0.45	0.38	0.25	0.50	0.38
B07	0.15	0.05	0.14	0.13	0.11
B08	0.10	0.05	0.08	0.05	0.07
B09	0.15	0.10	0.06	0.08	0.09
B10	0.23	0.09	0.18	0.11	0.14
Average	0.15	0.10	0.17	0.09	

Table A7 Mean Pt for each soil and time after extraction in test 5

Soil	Time after extraction				
	5 min	20 min	35 min	125 min	Average
A01	5.32	5.41	5.56	6.64	5.73
A02	3.21	3.41	3.43	3.75	3.45
A03	2.83	2.98	3.05	3.50	3.09
A04	1.89	2.02	2.03	2.18	2.03
B01	2.69	2.86	2.90	3.32	2.94
B02	6.49	6.77	7.12	8.04	7.10
B03	6.03	6.29	6.59	7.67	6.64
B04	5.77	5.97	6.27	7.62	6.41
B05	3.94	4.22	4.26	4.64	4.26
B06	6.56	7.52	7.71	8.88	7.67
B07	1.51	1.61	1.59	1.87	1.65
B08	1.43	1.42	1.43	1.59	1.47
B09	0.68	0.63	0.59	0.73	0.66
B10	2.55	2.85	2.71	2.92	2.76
Average	3.64	3.85	3.95	4.52	

Table A8 Mean Pt for each soil and separation method in test 6

Soil	Separation method			
	Centrifuge	Manual	Robot	Average
B01	3.18	3.03	2.23	2.82
B02	8.32	7.44	6.16	7.31
B03	8.13	6.68	5.66	6.82
B04	7.63	6.75	5.97	6.78
B05	5.23	4.56	4.43	4.74
B06	8.66	7.74	7.60	8.00
B07	2.08	2.00	1.91	2.00
B08	2.11	1.73	1.49	1.77
B09	1.04	0.93	0.88	0.95
B10	3.55	3.19	2.71	3.15
Average	4.99	4.40	3.91	

Table A9 Mean Pt for each soil and treatment in test 7

Soil	Treatment						
	A	B	C	D	E	F	Average
B02	7.85	7.80	6.56	8.22	8.38	8.02	7.81
B04	6.93	7.22	5.90	6.87	7.43	7.32	6.95
B06	7.81	8.17	6.62	7.73	8.22	8.15	7.78
B08	1.66	1.65	1.71	1.71	1.66	1.66	1.68
B10	3.17	3.12	3.21	3.23	3.24	3.21	3.20
Average	5.48	5.59	4.80	5.55	5.79	5.67	

Table A10 Mean of SE of Pt for each soil and treatment in test 7

Soil	Treatment						
	A	B	C	D	E	F	Average
B02	0.37	0.22	0.36	0.66	0.19	0.41	0.34
4	0.55	0.18	0.09	0.46	0.17	0.55	0.27
B06	0.46	0.37	0.21	0.52	0.69	0.42	0.42
B08	0.09	0.09	0.21	0.08	0.05	0.12	0.10
B10	0.13	0.07	0.03	0.07	0.03	0.18	0.07
Average	0.26	0.15	0.13	0.24	0.13	0.29	

Appendix 3 – Temperature plots

Contents and figure legends

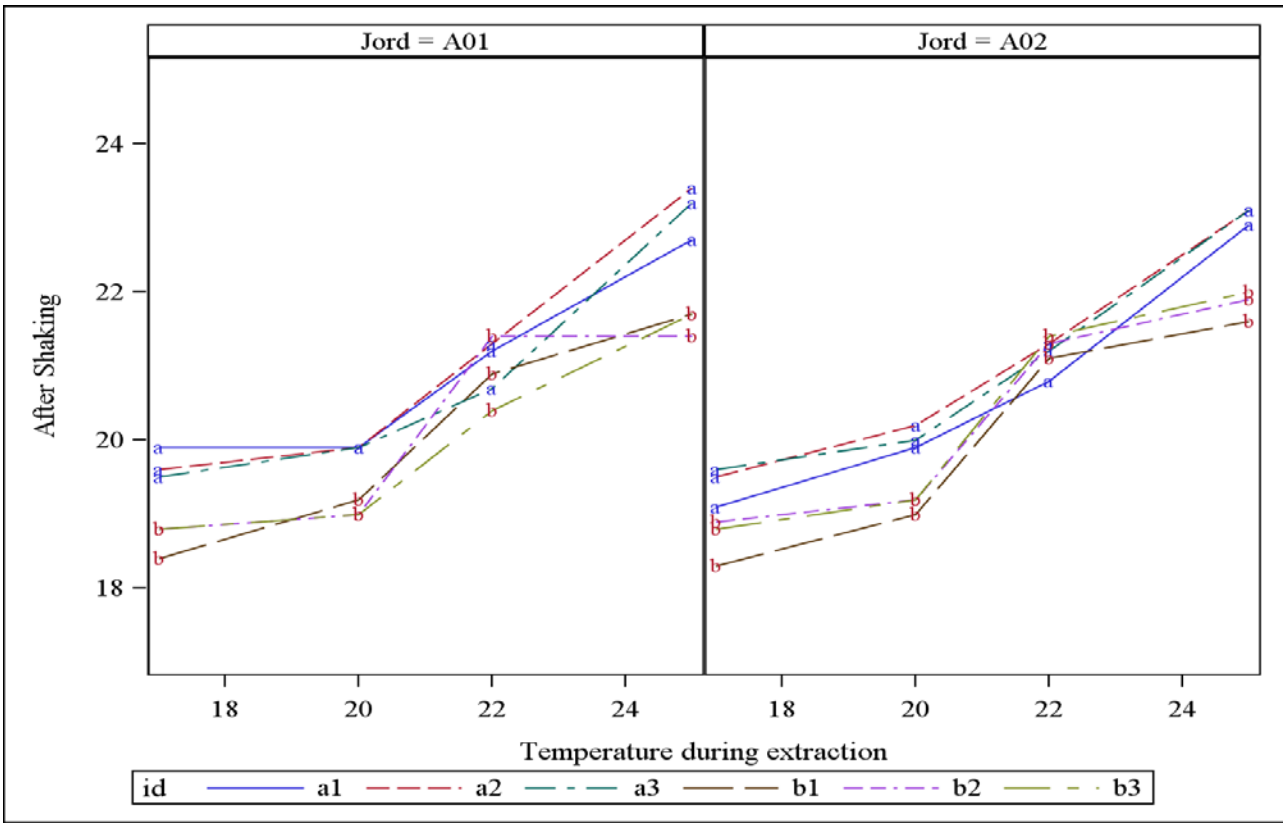
The appendix contains a plot for each soil showing the planned temperature during extraction and the measured temperature after shaking.

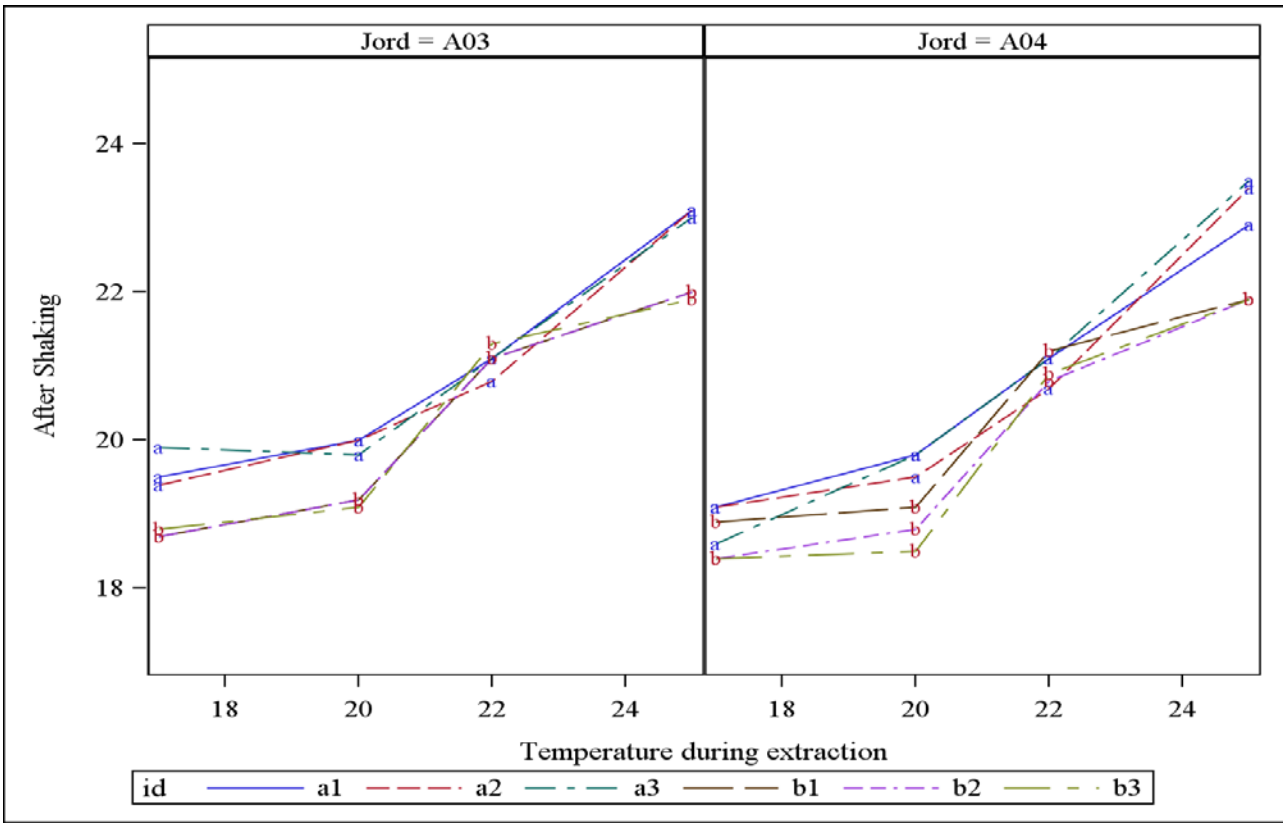
The following legends are used in all figures: the symbols *a* and *b* identify the run in which sample were included and the line type and the colour of the line connecting the point identify the actual sample.

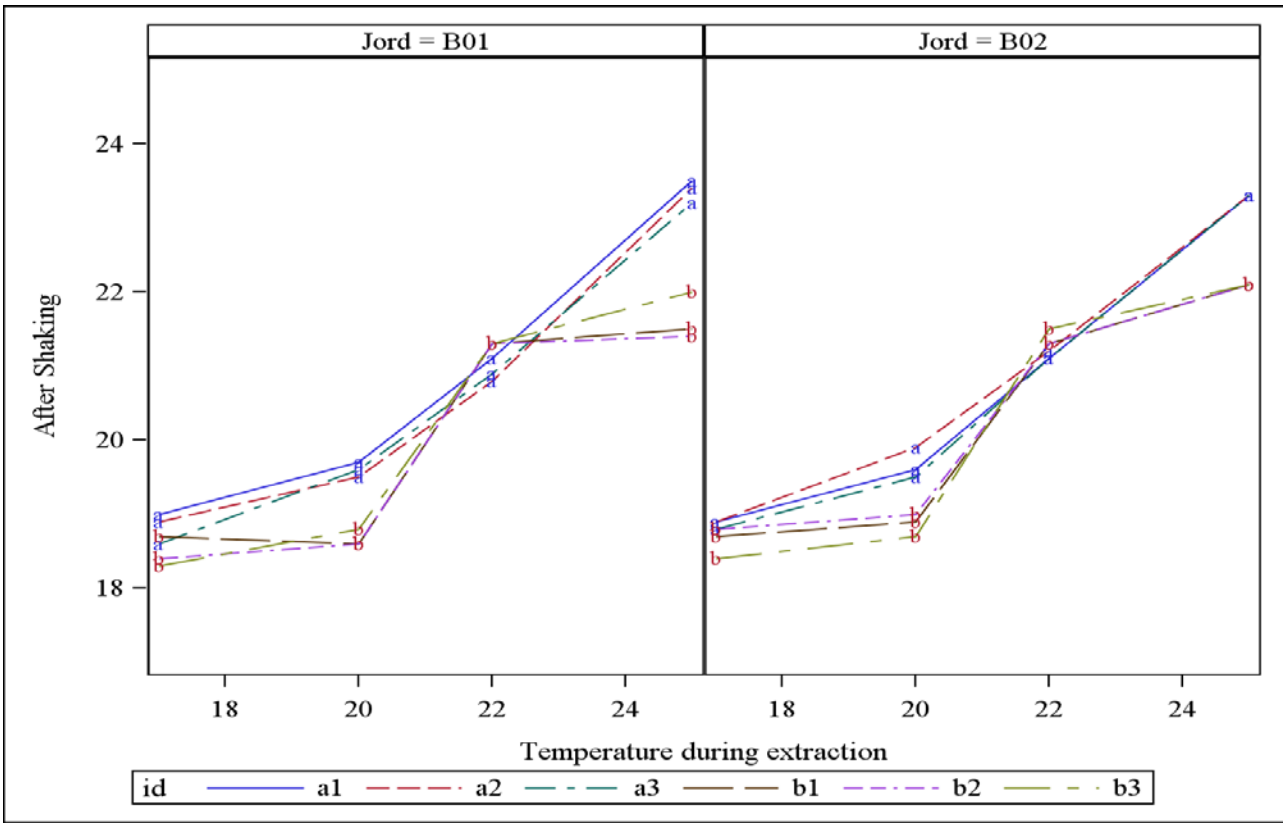
Comments

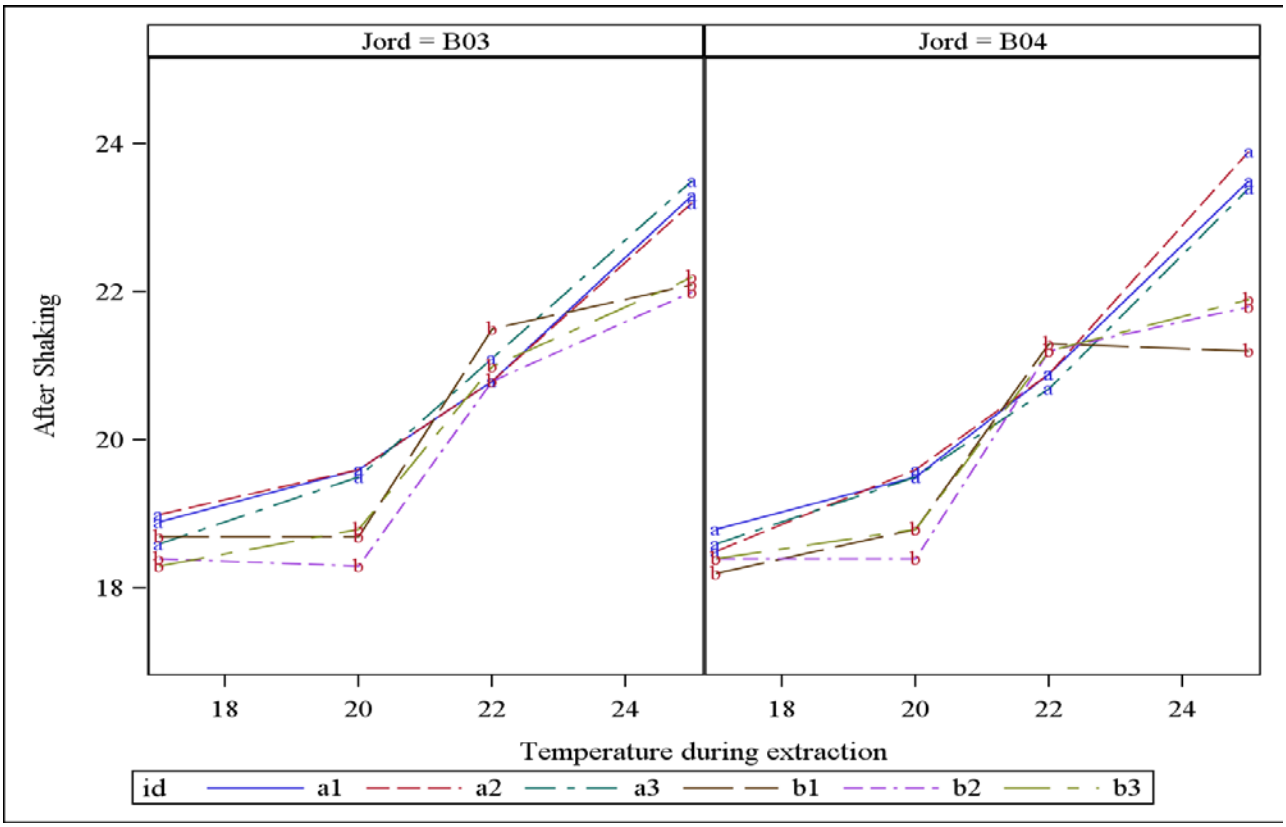
It was expected and intended that the temperature after shaking should not deviate from the initial pre-set temperature during extraction. However, deviations were found. E.g. for soil A01 the temperature after shaking was almost the same (20°C for a1 at both a planned temperature at 17°C and 20°C). A similar tendency is seen for the other two samples from the same run and planned temperatures.

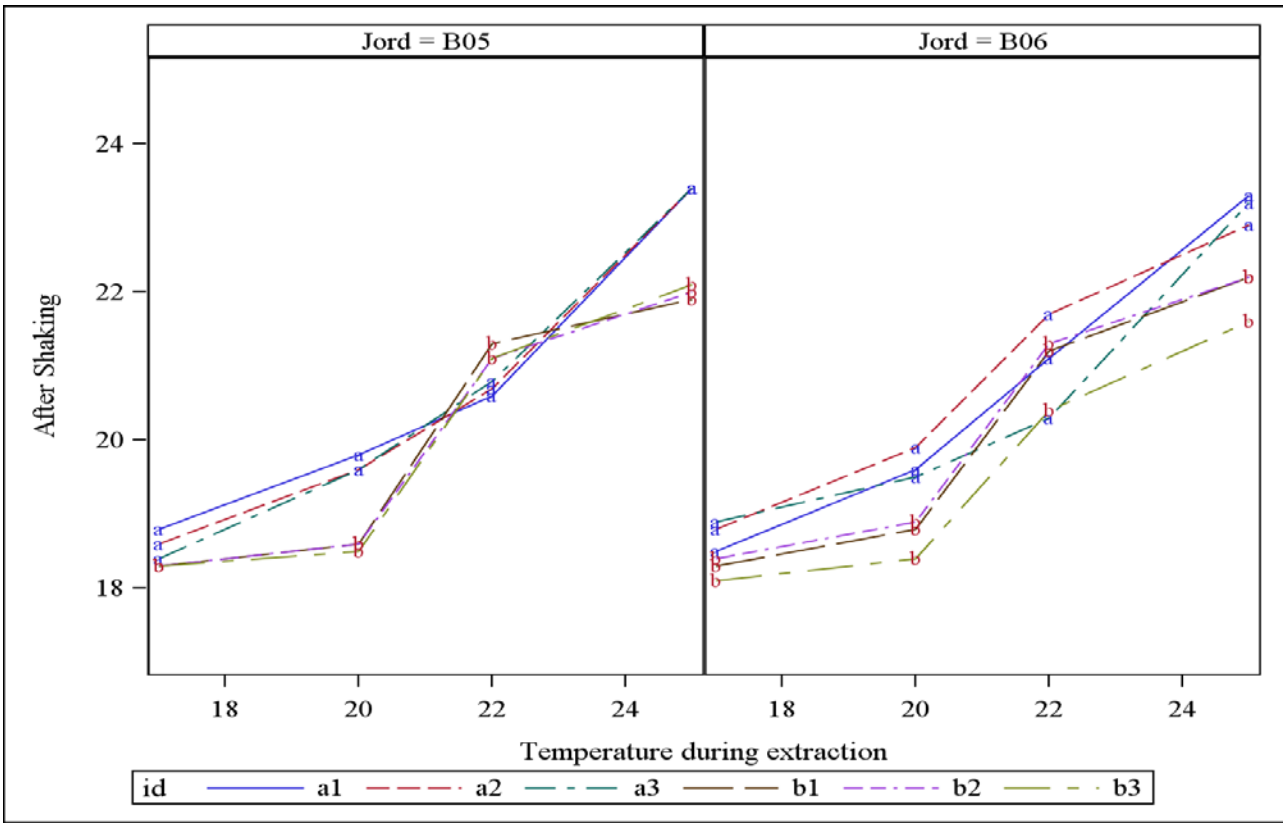
Another example is Soil A2 where the increase in temperature after shaking is almost proportional to the planned temperature during extraction for all samples at test *a* while the largest part of the increase for samples at test *b* occurs between planned temperature of 20°C and 22°C. Changes in the planned temperatures were seen for all other soils too.

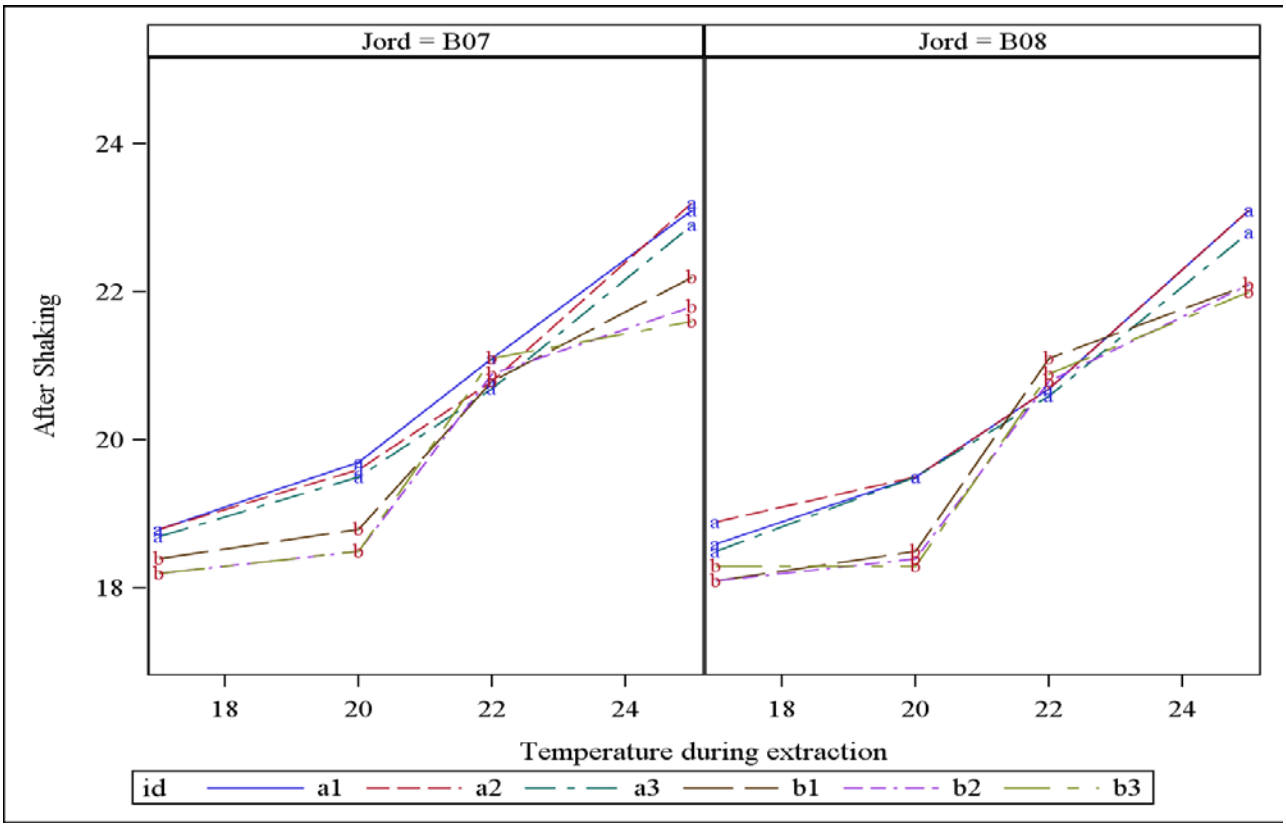

Jord = A01
Jord = A02
After Shaking
24
22
20
18
18
20
22
24
Temperature during extraction
id
a1
a2
a3
b1
b2
b3

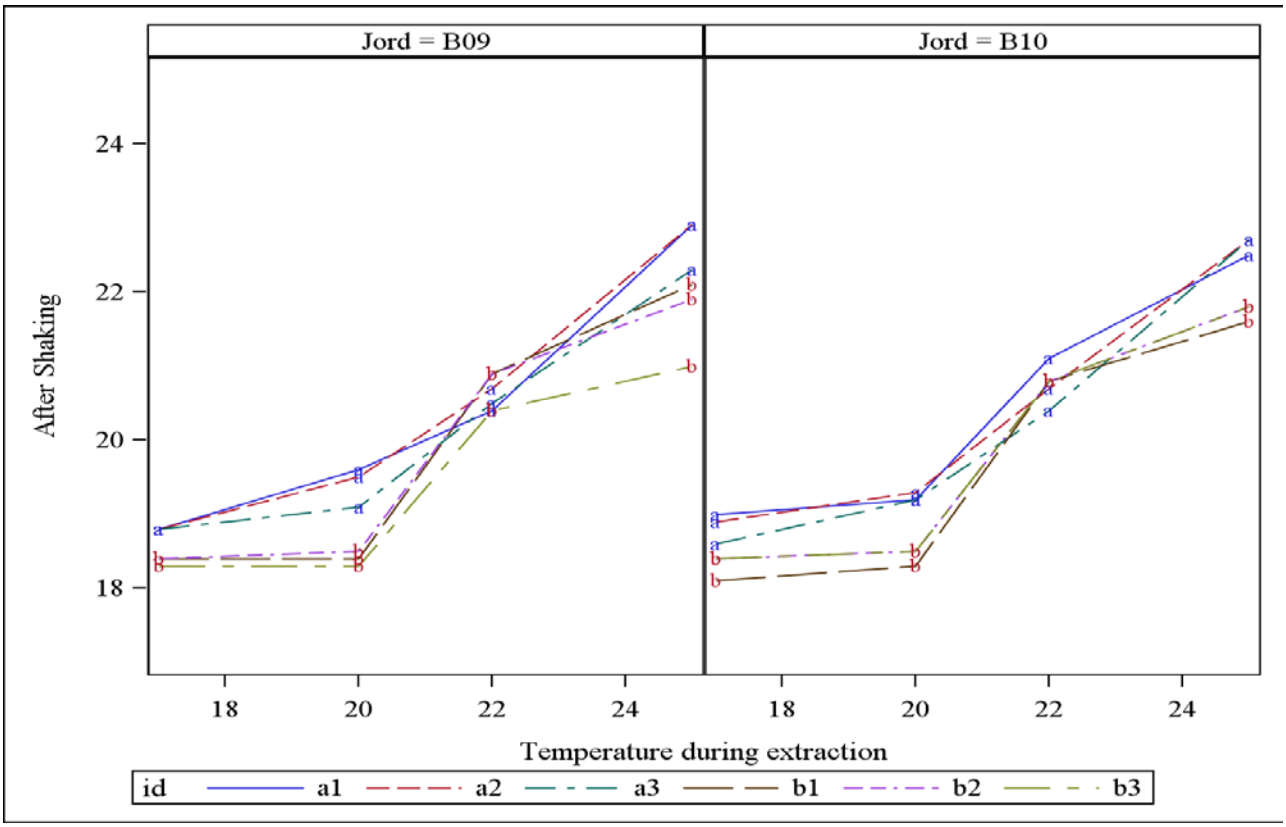

Jord = A03
Jord = A04
After Shaking
24
22
20
18
18
20
22
24
Temperature during extraction
id
a1
a2
a3
b1
b2
b3


Jord = B01
Jord = B02
After Shaking
24
22
20
18
18
20
22
24
Temperature during extraction
id
a1
a2
a3
b1
b2
b3


Jord = B03
Jord = B04
After Shaking
24
22
20
18
18
20
22
24
Temperature during extraction
id
a1
a2
a3
b1
b2
b3


Jord = B05
Jord = B06
After Shaking
24
22
20
18
18
20
22
24
Temperature during extraction
id
a1
a2
a3
b1
b2
b3


Jord = B07
Jord = B08
After Shaking
24
22
20
18
18
20
22
24
Temperature during extraction
id
a1
a2
a3
b1
b2
b3


Jord = B09
Jord = B10
After Shaking
24
22
20
18
18
20
22
24
Temperature during extraction
id
a1
a2
a3
b1
b2
b3

SUMMARY

The Olsen phosphorus (P) soil test, known in Denmark as “Ptallet” or “fosfortallet”, has been used for decades for P fertilizer recommendations. Today it is also used to device legislative thresholds for application of P to farmers fields. It is therefore unfortunate that the test results of this method vary significantly and systematically between laboratories and over time. Consequently, there has been several initiatives to document this and suggest ways to increase the robustness of the analysis. This report is the outcome of a project commissioned by the Environmental Protection Agency to quantify the importance of key elements in the Danish protocol for the Olsen P analysis and formulate a final concise protocol suitable for implementation in commercial soil laboratories. An advisory board consisting of representatives from three soil P testing commercial soil laboratories, SEGES and experts from Copenhagen and Aarhus Universities supervised this project. The results of the tests, recommendations of the advisory board and the final version of the protocol for the Olsen P analysis is included in this report.