Assessment and Analysis of Designs for Fertilizer Experiments

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Abstract

This study aimed to present a methodology for statistical analysis of fertilizer experiments conducted in greenhouse conditions and on the field with different soil types and contrasting soil-physical characteristics. Experimental designs will be used in the planning of multifactorial experiments that allow the assessment of actions and interactions of more than three factors, varying on three or more levels. Assessment of the information content of two types of designs for fertilizer trials shows their suitability for application in a basic research project. Variance and regression analyzes are illustrated with results from pot experiment and field experiment with four nutrients - nitrogen, phosphorus, potassium, and silicon. Corresponding algorithms a given for processing of the results.

Key words: fertilizer experiments, experimental designs, statistical analysis

Introduction

Agricultural experiments have their peculiarities and differ substantially from industrial experiments. The first feature pointed out by Peregudov (1972) is that in field experiments there is a significant drift in both time and space, and the noise level is very high. As a rule, the method of steepest descent and sequential experimental design is not applicable. For this purpose, sufficiently extensive and representative experimental data obtained from single or, multiple serial homogeneous experiments are necessary.

The second feature of agricultural experiments is that they are used to study ill-defined (poorly conditioned) processes and systems whose mathematical expression is *a priori* unknown. As a result, the researcher is in a state of uncertainty and cannot make a conclusion regarding “the true model”. Klevtsov and Sadovski (1981) emphasize that experimental designs must allow the simultaneous solution of two problems: the problem of discriminating between several alternative models and the problem of the optimal assessment of their parameters. Therefore such designs must contain 5 – 7 factor levels.

The objects of agricultural research are ill-defined also as a consequence of their multidimensional nature, i.e. they depend on a very large number of defining factors. And so, the third special feature of agricultural experiments is that they need to be multifactorial. Researchers often want to include 5 – 10 or more factors in their designs (Sadovski, 2018).

The starting and main point of field trials is the zero point (control). In almost all cases when quantitative factors vary, the part of interest is the positive quadrant of a hypersphere centered at the origin. This fourth feature of agricultural experiments rejects the principle of rotatability.

Many agricultural experiments detect interactions between the factors and the interactions of high order are almost always statistically not significant. This fifth feature requires that first-order interactions be evaluated.

Finally, it is well known that conducting field trials is quite difficult and if we increase the number of factors and levels, the practical implementation of factorial schemes would become impossible. Hence the sixth feature of agricultural experiments is that they need to use designs close to saturated designs. It is sensible to assume that such designs need to have at least one degree of freedom for each term in the model and one for error assessment.

Considering these six specific features of the experiment in agricultural research, and evaluation of the feasibility of the different types of multifactorial designs can be made. The study aimed to present a methodology for statistical analysis of fertilizer experiments conducted in greenhouse conditions and on the field. Variance and regression analyzes are illustrated with results from pot experiment and field experiment with four nutrients - nitrogen (N), phosphorus (P), potassium (K), and silicon (Si).

Materials and Methods

Experimental designs will be used in the planning of multifactorial experiments that allow the assessment of actions and interactions of more than three factors, varying on three or more levels. The work program of the project envisages the use of schemes of this kind (Petkova et al., 2019). The application of the following experimental designs is discussed:

A. Design, which is 1/2 replication of a 2^4 factor scheme with added control variant.

Table 1. *Design of* $1/2 \times 2^4$ *type.*

Variant	Factors			
No	A	B	C	D
1	1	1	1	1
2	2	1	1	2
3	1	2	1	2
4	2	2	1	1
5	1	1	2	1
6	2	1	2	2
7	1	2	2	2
8	2	2	2	1
9	0	0	0	0

This design will be used in conducting the field fertilizer trials with two replications. In the first 8 design variants, the triple interactions are ignored. It can assess the main effects of factors and their two-factor interactions (Davies, 1954; Nalimov & Chernova, 1965). Since this is a half-replicate, each comparison measures a pair of effects, the pairs being

(A, BCD); (B, ACD); (C, *ABD); (D, ABC); (AB, CD); (AC, BD); (AD, BC)*

Regression analysis also includes control variant No. 9. So the number of variants for this design is equal to 9. Here is a program for regression analysis of the results of the example implemented with MATLAB. The program code is applicable also with the free software GNU Octave (Eaton et al., 2020).

```
X = [ ];  # input design matrix
Y = [ ];  # input observed dependent variable
n = 18;  # number of variants with replications;
m = 4;  # number of terms in regression equation;
[n, m] = size(X);
b = (X'*X)^-1*X'*Y;          # regression coefficients
Yc = X * b;                  # calculated dependent variable
SSE = Y'*Y-b'*X'*Y;          # error sum of squares
SSR = b'*X'*Y;               # regression sum of squares
SST = Y'*Y;                  # total sum of squares
R2 = SSR/SST;                # coefficient of determination
sigma = sqrt(SSE/(n-m));     # standard error
F = SSR/(n*sigma^2);         # F test
pf = fpdf(F,m,n-m);          # probability of F value
T = (X'*X)^-1;               # inverse matrix of sum of squares and products
for j = 1:m
t(j) = b(j)/(sigma*sqrt(abs(T(j,j))));
pt(j) = tpdf(t(j),n-m);
end;
t;                           # Student t
pt;                          # probability of t
```

B. Experimental design of composite type with 4 factors at 5 levels.

Table 2. *Design of type 4C1648*

Variant	Factors			
No	A	B	C	D
1	0	0	0	0
2	0	2	2	2
3	4	2	2	2
4	2	0	2	2
5	2	4	2	2
6	2	2	0	2
7	2	2	4	2
8	2	2	2	0
9	2	2	2	4
10	2	2	2	2
11	3	3	1	1
12	3	1	3	1
13	3	1	1	3
14	1	3	3	1
15	1	3	1	3
16	1	1	3	3

Concerning the six features of agricultural experiments mentioned in the introduction, a new family of multifactorial designs for the construction of dynamic yield models depending on the manageable yield-forming quantitative factors was developed (Sadovski, 1984).

The proposed experimental designs are composite and their construction can be illustrated with k = 4 number of factors. Each of the factors has 5 levels coded as 0, 1, 2, 3, and 4. The basic configuration consists of 2k star points:

(0, 2, 2, 2); (4, 2, 2, 2); (2, 0, 2, 2); (2, 4, 2, 2);

(2, 2, 0, 2); (2, 2, 4, 2); (2, 2, 2, 0); (2, 2, 2, 4);

one central point (2, 2, 2, 2) and one zero point (0, 0, 0, 0).

To evaluate the two-factor interactions of the first order, this design is combined with k(k-1)/2 points, which are part of Rechtschafner's (1967) saturated designs with two levels:

(3, 3, 1, 1); (3, 1, 3, 1); (3, 1, 1, 3);

(1, 3, 3, 1); (1, 3, 1, 3); (1, 1, 3, 3).

This configuration resembles a kite and provides degrees of freedom for estimation of the constant term, 4 linear and 4 quadratic terms, 6 interactions, and the error.

The proposed design in Table 2 will be used in conducting of greenhouse experiments. This design with a number of factors - 4 and a number of levels - 5, allows obtaining regression models in the form of a complete second-degree polynomial or other function (linear or nonlinear) with up to 15 unknown regression coefficients. ANOVA can evaluate first-order main actions and two-factor interactions.

Regression model such as second-degree polynomial

$$y = a_0 + \sum_{i=1}^{k} a_i X_i + \sum_{i=1}^{k} a_i X_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} a_{ij} X_1 X_j$$

or other functions (linear or nonlinear) of the factors $y = \varphi(X_1, X_2, ..., X_k)$ can be fitted with unknown regression coefficients whose number does not exceed (k+1)(k+1)/2.

In the case of multiple linear models, the regression analysis can easily be performed after Gram-Schmidt orthogonalization. For example, the matrix presented in Table 2 is transformed using the procedure described by Draper and Smith (1973). When the columns of the *X* matrix are almost dependent, the *X'X* matrix will be almost singular and difficulties in inversion, including large round-off errors, are likely.

As a result, the following regression equation is obtained

$$y = b_0 + \sum_{j=1}^{n} b_j z_j,$$

where z_j are orthogonal variables, linear combinations of X_j. The information matrix *Z'Z* and its inverse $(Z'Z)^{-1}$ are diagonal.

After determination of the coefficients b_j by the formula

$$b = (Z'Z)^{-1} Z'y$$

a backward substitution is performed in the regression equation.

The corresponding estimation of the values of the dependent variable is

$$\hat{y} = Zb.$$

It should be noted that the use of the described composite designs in agricultural research is connected to the application of the two main principles of experimental planning – repetition and randomization.

The program for the regression analysis of the results of pot experiment, implemented with MATLAB differs from that for the field fertilizer trials. The first two statements differ, reflecting the size of matrix *X*. So the number of variants for this design *n* is equal to 16 and number of the terms *m* depends on the type of multiple linear regression equation. Also, the matrix *X* is transformed into matrix *Z* by orthogonalization.

```
n = 16;  # number of variants
m = 4;  # number of orthogonal polynomials
[n, m] = size(X);
Z = zeros(n, m);
R = zeros(m, m);
for j = 1:m
v = X(:,j);
for i = 1:j-1
R(i,j) = Z(:,i)'*X(:,j);
v = v - R(i,j)*Z(:,i);
end;
R(j,j) = norm(v);
Z(:,j) = v/R(j,j);
end;
b = (Z'*Z)^-1*Z'*Y;          # regression coefficients
Yc = Z * b;                  # calculated dependent variable
SSE = Y'*Y-b'*Z'*Y;          # error sum of squares
SSR = b'*Z'*Y;               # regression sum of squares
SST = Y'*Y;                  # total sum of squares
R2 = SSR/SST;                # coefficient of determination
sigma = sqrt(SSE/(n-m));     # standard error
F = SSR/(n*sigma^2);         # F test
pf = fpdf(F,m,n);            # probability of F value
T = (Z'*Z)^-1;               # inverse matrix of sum of squares and products
for j = 1:m
t(j) = b(j)/(sigma*sqrt(abs(T(j,j))));
pt(j) = tpdf(t(j),n-m);
end;
t;                           # Student t
pt;                          # probability of t
```

The application of the presented algorithms is presented with two examples.

Results and Discussion

The mathematical and statistical analysis of the experimental results is carried out by several sequential methods. Analysis of variance (ANOVA) makes it possible to calculate the error of the arithmetic mean over the whole experiment, which is used to evaluate all possible differences between the variants tested. A useful property of ANOVA is that its estimates are consistent even with deviations from the normal (Gaussian) distribution. The next step is finding regression equations of different types - complete second-degree polynomial, polynomial with square roots, or other function (linear or nonlinear).

To illustrate the way of analysis of data obtained after the field fertilizer trials here is given as Example 1. Let start with the following experimental data. All calculations could be performed on an Excel table.

Table 3. *Example with data from Table 10.23 (Davis, 1954)*

Variant	N	P	K	Si	Yaver	I repl.	II repl.
1	-1	-1	-1	-1	107	106	108
2	1	-1	-1	1	114	113	115
3	-1	1	-1	1	122	121	123
4	1	1	-1	-1	130	129	131
5	-1	-1	1	1	106	105	107
6	1	-1	1	-1	121	120	122
7	-1	1	1	-1	120	120	120
8	1	1	1	1	132	131	133

Table 4. *Calculation of effects*

Variant	N*Y	P*Y	K*Y	N*P*K*	SIGN(N)*P*	SIGN(N)*K*	SIGN(P)*K*
1	-106	-106	-106	-106	106	106	106
2	113	-113	-113	113	-113	-113	113
3	-121	121	-121	121	-121	121	-121
4	129	129	-129	-129	129	-129	-129
5	-105	-105	105	105	105	-105	-105
6	120	-120	120	-120	-120	120	-120
7	-120	120	120	-120	-120	-120	120
8	131	131	131	131	131	131	131
1	-108	-108	-108	-108	108	108	108
2	115	-115	-115	115	-115	-115	115
3	-123	123	-123	123	-123	123	-123
4	131	131	-131	-131	131	-131	-131
5	-107	-107	107	107	107	-107	-107
6	122	-122	122	-122	-122	122	-122
7	-120	120	120	-120	-120	-120	120
8	133	133	133	133	133	133	133
Total	84	112	12	-8	-4	24	-12
Effects	21	28	3	-2	-1	6	-3

Table 5. *Analysis of variance*

Source	Total	Effects	SSQ	SSQ %	DF	F	p
N	84	21.00	441.0	33.975	2	31.500	0.0020
P	112	28.00	784.0	60.401	2	56.000	0.0008
K	12	3.00	9.0	0.693	2	0.643	0.2894
Si	-8	-2.00	4.0	0.308	2	0.286	0.5077
NP,KSi	-4	-1.00	1.0	0.077	2	0.071	0.8185
NK,PSi	24	6.00	36.0	2.773	2	2.571	0.0657
NSi,PK	-12	-3.00	9.0	0.693	2	0.643	0.2894
Factors	208	52	1284.0	98.921	14	91.714	0.0004
Error			14.0	1.079	1		
Total			1298.0	100.000	15		

The error variance is 5.408 and on this basis N, P, and interaction (NK,PSi) is significant. The regression model, which corresponds to the data of the example and includes the control variant, has the form

$Y = b_0+b_1N+b_2P+b_3NK$

The input matrix of this model is given below:

X = [1 1 1 1;1 2 1 2;1 1 2 1;1 2 2 2;1 1 1 2;1 2 1 4;1 1 2 2;1 2 2 4;1 0 0 0;
1 1 1 1;1 2 1 2;1 1 2 1;1 2 2 2;1 1 1 2;1 2 1 4;1 1 2 2;1 2 2 4;1 0 0 0].

Dependent variable is input by replications:

Y = [106 113 121 129 105 120 120 131 99 108 115 123 131 107 122 120 133 101].

n = 18; # number of variants with replications;

m = 4; # number of terms in regression equation;

Here are the results of the regression analysis from Example 1. The regression coefficients are denoted by b with corresponding t-criterion values in Table 6.

Table 6. *Regression results of Example 1.*

Coeff.	b	t	p(t)
b0	94.741	38.640	0.0000
b1	4.306	1.664	0.1012
b2	10.056	5.959	0.0000
b3	1.500	1.148	0.1997

On Table 7 are given input values of dependent variable Y, corresponding calculated by regression values Yc and their difference O-C. Estimated coefficient of multiple determination R^2 = 0.999 and standard error = 3.424.

Table 7. *Comparison of the input and calculated values from Example 1.*

Y	Yc	O - C
107	109.463	-2.46
114	116.019	-2.02
122	119.519	2.48
130	131.741	-1.74
106	109.463	-3.46
121	116.019	4.98
120	119.519	0.48
132	131.741	0.26
100	98.519	1.48

The design of Table 2 is applicable to multifactorial fertilizer experiments in greenhouse conditions. The vegetation experiment will be carried out in plots with 3 kg soil in three replications. Soils from the same experimental fields will be used.

To illustrate the way of analysis of data obtained from the greenhouse trials here is given as Example 2.

Table 8. *Example of experimental data.*

Variants	N	P	K	Si	I repl.	II repl.	III repl.
1	0	0	0	0	50	52	48
2	0	2	2	2	60	62	58
3	4	2	2	2	120	125	115
4	2	0	2	2	70	71	69
5	2	4	2	2	115	120	110
6	2	2	0	2	80	82	78
7	2	2	4	2	114	116	112
8	2	2	2	0	90	92	88
9	2	2	2	4	120	125	115
10	2	2	2	2	90	92	88
11	3	3	1	1	100	105	95
12	3	1	3	1	90	92	88
13	3	1	1	3	95	95	95
14	1	3	3	1	80	82	78
15	1	3	1	3	80	82	78
16	1	1	3	3	90	92	88

Two-dimensional surfaces of mean Y values in relation to N x P variants and N x Si variants are presented in Figure 1 and Figure 2.

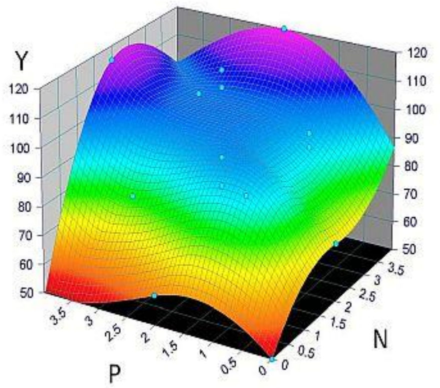

Fig. 1. *Surface N x P*

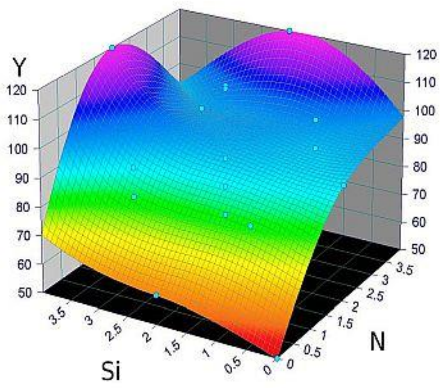

Fig. 2. *Surface N x Si*

From these figures, it can be seen with the naked eye that the increasing rates of nitrogen, phosphorus, and silicon give a significant increase in the dependent variable Y.

The statistical processing of data by means of the analysis of variance for the new design can be performed using orthogonal polynomials, which can evaluate main effects and two-factor interactions. The statistical analysis for this design is illustrated with data from Table 8

Let the dependent variable (yield) be expressed with the model

$$Y_i = \alpha_0\, \varphi_0(T_i) + \alpha_1\, \varphi_1(T_i) + \ldots + \alpha_k\, \varphi_k(T_i) + \varepsilon_i, \quad (i = 1, 2, \ldots, n),$$

where n is the number of treatment combinations; $\alpha_0, \alpha_1, \ldots, \alpha_k$ are regression coefficients; $\varphi_0, \varphi_1, \ldots, \varphi_k$ are orthogonal polynomials of the independent variables N, P, K, and Si. With T_i designates the consecutive treatment combinations in the design. It is known that the orthogonal polynomials satisfy the conditions

$$\sum_{i=1}^{n} \varphi_r(T_i).\varphi_s(T_i) = 0, \quad (r, s = 0, 1, 2, \ldots, k; r \neq s).$$

The total sum of squares is decomposed into individual components as follows (Kendall and Stuart, 1961)

$$\sum_{i=1}^{n} (Y_i - \bar{Y})^2 = \sum_{r=1}^{k} \frac{[\sum_{i=1}^{n} \varphi_r(T_i) Y_i]^2}{\sum_{i=1}^{n} \varphi_r(T_i)}.$$

Since the levels of the separate factors in the design have equidistant values: *x* = 0, 1, 2, 3, 4, the explicit expression of Laguerre orthogonal polynomials (Abramowitz & Stegun, 1964) up to second degree for each factor is

φ_0 = 1,

φ_1 = -x + 1,

φ_2 = (x2 - 4x + 2)/2.

The degrees of freedom for the variance of the treatments are distributed as follows: one for each component up to third-degree for the main effects of the factors and one for each of the four components for evaluation of the two-factor interactions.

When *x* is substituted with the levels of the factors N, P, K, and Si for the consecutive treatments in Table 2, the values of the orthogonal polynomials for the linear and quadratic components of the factors are obtained.

$\varphi_0 = 1$; $\varphi_1 = N_L$; $\varphi_2 = N_Q$; $\varphi_3 = P_L$; $\varphi_4 = P_Q$; $\varphi_5 = K_L$; $\varphi_6 = K_Q$; $\varphi_7 = Si_L$; $\varphi_8 = Si_Q$.

The values of the pair-wise interactions between the factors are found via term-by-term multiplication.

$\varphi_9 = N_L.P_L$; $\varphi_{10} = N_L.K_L$; $\varphi_{11} = N_L.Si_L$; $\varphi_{12} = P_L.K_L$; $\varphi_{13} = P_L.Si_L$; $\varphi_{14} = K_L.Si_L$.

The sums of squares of the orthogonal polynomials

$$\sum_{i=1}^{n} \varphi_r^2(T_i), \quad (r = 1, 2, ..., 21)$$

for all treatments in one replication of the design are constants and is equal to 16.

The working formulas for calculation of the sums of squares of the orthogonal components are expressed as

$$SS_{(r)} = [\sum_{i=1}^{n} \varphi_r(T_i) Y_i]^2 / \sum_{i=1}^{n} \varphi_r^2(T_i)$$

or $SS_{(r)} = \alpha_r^2 \sum_{i=1}^{n} \varphi_r^2, \quad (r = 1, 2, ..., 21).$

So we have the following values:

$SS_{(1)} = 3. -55,8974^2.16 = 149977,7328$

$SS_{(2)} = 3. -16,5067^2.16 = 13078,6101$

. . . .

$SS_{(14)} = 3. -0,7393^2.16 = 26,2337$

The multiplier 3 in the above calculations reflects the three replications of the treatments in the experiment. The results of the calculations are presented in Table 9 after combining the sums of squares of the respective components for the factors and interactions, as follows:

$SS_V = SS_N + SS_P + SS_K + SS_{Si} + SS_{NP} + SS_{NK} + SS_{NSi} + SS_{PK} + SS_{PSi} + SS_{KSi}$;

$SS_N = SS_{(1)} + SS_{(2)}$; $SS_P = SS_{(3)} + SS_{(4)}$;

$SS_K = SS_{(5)} + SS_{(6)}$; $SS_{Si} = SS_{(7)} + SS_{(8)}$;

$SS_{NP} = SS_{(9)}$; $SS_{NK} = SS_{(10)}$; $SS_{NSi} = SS_{(11)}$;

$SS_{PK} = SS_{(12)}$; $SS_{PSi} = SS_{(13)}$; $SS_{KSi} = SS_{(14)}$.

The error sum of squares is calculated by subtraction

$SS_E = SS_T - SS_V$.

The mean squares (variances) for each row in the table of the analysis of variance are obtained by dividing the sum of squares by the degrees of freedom. The experimental values of Fisher’s criterion are calculated in the usual manner through division by the error variance of the respective variance in each row. The statistical significance is verified using the F-test, as usual.

Table 9. *Analysis of variance of Example 2.*

Source of variation	SS	SS%	DF	MS	F	p%
Total	409938.00	100.00	47	-	-	-
Variants	300187.00	73.23	14	21441.93	6.4472	<0.1
N	163056.34	39.78	2	81528.17	24.5139	<0.1
P	41538.50	10.13	2	20769.25	6.2449	<1
K	22276.16	5.43	2	11138.08	3.3490	<5
Si	57429.83	14.01	2	28714.91	8.6340	<0.5
N.P	15796.41	3.85	1	15796.41	4.7497	<5
N.K	60.20	0.01	1	60.20	0.0181	-
N.Si	29.56	0.01	1	29.56	0.0089	-
P.K	1302.07	0.32	1	1302.07	0.3915	-
P.Si	3.81	0.00	1	3.81	0.0011	-
K.Si	26.23	0.01	1	26.23	0.0079	-
Error	109751.00	26.77	33	3325.79	-	-

From the analysis of variance table is evident that all main effects of nitrogen, phosphorus, potassium, and silicon are significant. Only the interaction of nitrogen and phosphorus should be taken into account.

The results of the regression analysis from Example 2 are presented in Table 10. The regression coefficients of all orthogonal polynomials are denoted by alpha with the corresponding t-criterion.

Table 10. *Regression results of Example 2.*

Term	alpha	t
Const	361.0000	0.0001
N_L	-55.8974	0.0044
N_Q	-16.5067	0.0437
P_L	-28.6324	0.0160
P_Q	-6.7496	0.1553
K_L	-21.0467	0.0284
K_Q	4.5955	0.2141
Si_L	-23.5894	0.0230
Si_Q	25.2982	0.0202
$N_L.P_L$	18.1406	0.0371
$N_L.K_L$	-1.1199	0.3094
$N_L.Si_L$	0.7848	0.3139
$P_L.K_L$	5.2083	0.1959
$P_L.Si_L$	-0.2816	0.3177
$K_L.Si_L$	-0.7393	0.3144

On Table 11 are given input values of dependent variable Y, corresponding calculated by regression values Yc and their difference O-C. Estimated coefficient of multiple determination is R^2 = 0.999 and standard error = 6,587.

Table 11. *Comparison of the input and calculated values from Example 2.*

Y	Yc	O - C
50	50.87	-0.87
60	57.95	2.05
120	117.60	2.40
70	69.25	0.75
115	111.75	3.25
80	77.59	2.41
114	112.61	1.39
90	84.88	5.12
120	120.82	-0.82
90	92.08	-2.08
100	104.89	-4.89
90	92.53	-2.53
95	95.08	-0.08
80	83.74	-3.74
80	81.82	-1.82
90	90.26	-0.26

The next step of the analysis is to find the optimum of the nutrient elements N, P, K, and Si.

Conclusion

A methodology is presented for statistical analysis of fertilizer experiments conducted in greenhouse conditions and on the field with different soil types. Assessment of the information content of two types of designs for fertilizer trials and their suitability for application in a basic research project is presented. The objects of discussion are the design of $1/2 \times 2^4$ type and design of type ${}_4C^{16}{}_{48}$. The common property of both designs is that they allow studying the influence of four nutrients N, P, K, and Si. The difference is that the first allows estimation of the main effects and two-factor interactions with two levels of the nutrients. It is suitable for field fertilizer trials. The second design with a number of factors - 4 and number of levels - 5, allows obtaining regression models in the form of a complete second-degree polynomial or other function (linear or nonlinear) with up to 15 unknown regression coefficients.

Variance and regression analyzes are illustrated with results from pot experiment and field experiment with four nutrients. Corresponding algorithms code in MATLAB is given for processing of the results. Calculations of the two examples are presented to illustrate their application. The methodology will be applied with real data from greenhouse and field experiments conducted on two experimental fields with contrasting soil types.

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