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TWO–WAY PROFILE ANALYSIS WITH INTERACTION: AN APPLICATION TO MICROBIOLOGICAL EXPERIMENT

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Summary

In the paper the model of two–way profile analysis is applied to a microbiological experiment. There are given results of verification of appropriate hypothesis. Those results are also illustrated graphically.

Key words and phrases: profile analysis, multivariate statistical analysis, two–way experiment, microbiological experiment

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1. Introduction

In the Department of Soil Environment Sciences there were carried out a microfield experiment the aim of which was the assessment of the impact of produced composts from organic wastes on some properties of podzolic soil. In 2005 a pot experiment (microfield experiment) was set up in the field at the Skierniewice Experimental Station of Agriculture and Biology Faculty of the

Warsaw University of Life Sciences. Pots with of podzolic soil each were used in the experiment. Composts were produced from organic wastes such as: spent *Pleurotus ostreatus* wastes, the sewage sludge or decoction of molasses with addition of sawdust of pine or ash tree, with or without suplement effective microorganisms (EM) were used. Pot experiment was set up in three repetitions. The composts were added to the soil in conversion to 10 ton of dry matter per hectare. Hence there were obtained nine combinations of bases:

	Ingredients
1	Soil + Compost 1 (Sewage sludge + sawdust of pine tree)
2	Soil + Compost 2 (Sewage sludge + sawdust of ash tree)
3	Soil + Compost 3 (Sewage sludge + sawdust of pine tree + EM)
4	Soil + Compost 4 (Sewage sludge + sawdust of ash tree + EM)
5	Soil + Compost 5 (spent *Pleurotus ostreatus* wastes)
6	Soil + Compost 6 (spent *Pleurotus ostreatus* wastes + EM)
7	Control: Soil without compost
8	Soil + Compost 7 (decoction of molasses + sawdust of pine tree)
9	Soil + Compost 8 (decoction of molasses + sawdust of ash tree)

Dehydrogenase activity of soil which is the one of the most important indicator of microbiological activity of soil was measured. Dehydrogenase activity was investigated in the arable layer (0-20 cm of depth) of soil, five times during the vegetation season by three years (2005, 2006 2007): in May, June, July, August and September. Enzymes assays were performed in triplicate. Soil samples for enzymes activity were taken from the same pots in each month of experiments. Dehydrogenase activity in soil samples was measured by Casida's (1977) method. One unit of enzyme activity is defined as the amount of enzyme that produces 1.0 μg triphenylformazan per min.

It was expected that the experiment will give the answers to the following main questions.

1. Is there any influence of type of compost on the dehydrogenase activity of soil?
2. Does dehydrogenase activity depends on the year, i.e. does meteorological condition influence ont it?
3. Is there any “cooperation” between type of compost and meteorology?

In the statistical analysis the fact of the repeated measurements should be strongly exploit. Hence, the statistical method of the profile analysis was applied. Because there were five measurements on the same object, so dimension of the problem is five. There are also two factors influencing on results: type of compost added to the soil and year. Hence, the model of a two–way profile analysis was applied to the experiment. Mathematical technicalities of the analysis are presented in Zieliński (2009). In the next section numerical results are presented along with appropriate conclusions.

2. Analysis

The linear model for observations obtained in the experiment is as follows

$$y_{ijlh} = \mu_h + \alpha_{ih} + \tau_{jh} + \eta_{ijh} + \varepsilon_{ijlh},$$

for $i = 1,\ldots,k$, $j = 1,\ldots,c$, $l = 1,\ldots,m$ and $h = 1,\ldots, p$, $p > 1$. Here *k* is the number of levels of the first treatment, *c* is the number of levels of the second treatment, *m* is the number of independent vectors of observations in each combination and $p > 1$ is the number of random variables observed per cell, i.e. the dimension of observed vector. In the model, μ_h is a general mean of *h*th response, α_{ih} is an effect of *i*th treatment on *h*th response, τ_{jh} is an effect of *j*th treatment on *h*th response, η_{ijh} is an effect of interaction of *i*th and *j*th treatments on *h*th response and ε_{ijlh} is an usual normal random variable term.

In the considered experiment the first treatment was fertilizer applied on $k = 9$ levels denoted by $F_1,\ldots,F_9$. The second treatment was year. Here there are $c = 3$ levels denoted by Y_1, Y_2, Y_3. The dimension of the observation vectors was $p = 5$, and repetitions $m = 4$. In all analysis significance level was $\alpha = 0.05$.

Tests of the parallelism of profiles

We start the analysis with testing the parallelism of profiles of all treatments. The value 0.2181 of the appropriate test statistic was obtained, and the comparison of that value with $F(0.05,22,50) = 1.7588$ shows that the hypothesis is not rejected. It means that in average applied treatments has similar affect on the investigated variate. Now we investigate those effect in details.

Let us start with comparison of general means. The appropriate test statistic takes on the value 0.0644. If we compare this value with $F(0.05,3,50) = 2.7900$ we may see that general means for consecutive months may be treated as equal.

As a corollary we obtain that soil and the external conditions were the same for all investigated pots.

In the next step, we perform tests for profiles parallelism of main factors as well as interactions.

Results of testing parallelism of profiles

Factor	t	w	F_{stat}	F_{crit}
Fertilizer F	4	50	149.445	2.5572
Year Y	2	50	63.7658	3.1826
Interactions $F \times Y$	12	50	85.6692	1.9515

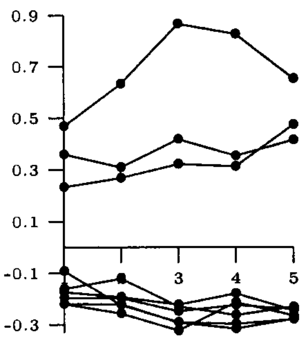

Fig. 1. Fertilizer profiles

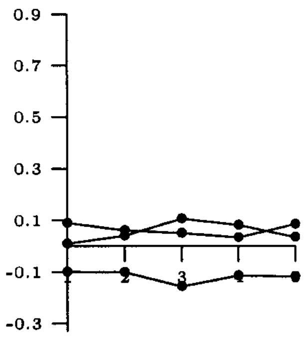

Fig. 2. Year profiles

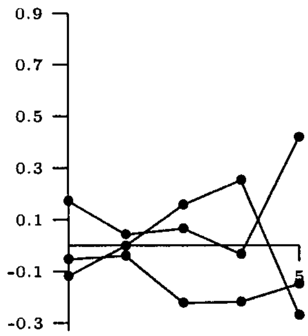

Fig. 3. Interaction profiles

Profiles of treatments effects are presented in the pictures: for fertilizer in the Fig. 1, for year in the Fig. 2 and for interaction in the Fig. 3. On the last picture there are shown only three profiles (for interactions $F_1 \times Y_1$, $F_1 \times Y_2$ and $F_1 \times Y_3$), because showing all the 27 profiles will do the figure unreadable.

In the Fig. 1 it easily seen that profiles are not parallel what means that dynamics of dehydrogenase activity of soil depends on the applied fertilizer. Similar conclusions may be done for years and interactions.

Note that our analysis is only illustration of theory, so the names of profiles are not important. Hence, profiles in figures are not distinguished. The microbiological analysis of the experiment will be presented elsewhere.

Testing equal levels of effects of treatments

It is interesting if investigated factors affect on dehydrogenase activity of soil "ignoring" the fact of repeated measurements. Results of the appropriate test are shown in the table below.

Factor	t	w	F_{stat}	F_{crit}
Fertilizer F	7	53	925.8343	2.1881
Year Y	1	53	391.7707	4.0230
Interactions $F \times Y$	15	53	113.6907	1.8595

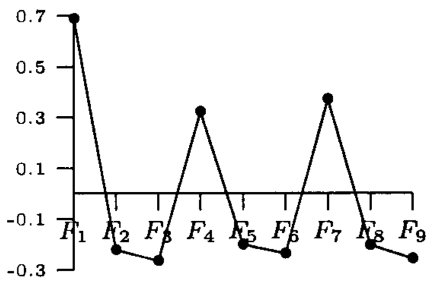

Fig. 4. Levels of fertilizers

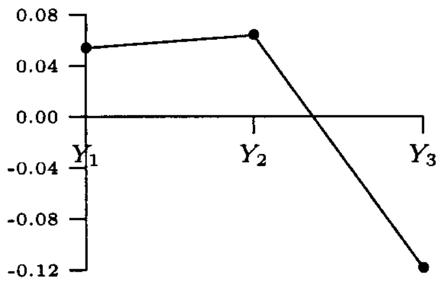

Fig. 5. Levels for years

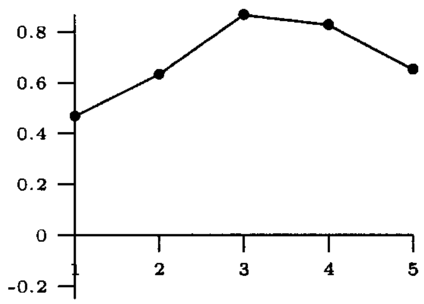

Fig. 6. First fertilizer

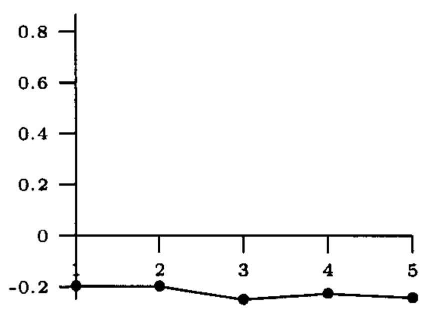

Fig. 7. Second fertilizer

In Figures 4 and 5 are shown effects of applying different fertilizers and different years respectively. It is seen that fertilizers F_1, F_4 and the control F_7 has positive general effect on dehydrogenase activity of soil while effects of others fertilizers were negative.

A negative effect of compost on dehydrogenase activity of soil in the third year of experiment was observed. It is connected with deficiency of organic substance in soil for microorganism development.

The picture of interactions is not presented here, but may be easily drawn using results given in Appendix.

Equal response

In this part of the analysis we want to check whether effects of treatments are the same in months. In other words, we ask if profiles for levels of investigated factors are horizontal. Results are shown in the table 1. By the symbol √ are denoted those levels of treatments profiles of which are horizontal. In the Figures 6 and 7 are shown profiles of levels of first two levels of the first factor i.e. fertilizer.

4. Concluding remarks

In the paper there are shown results of a microbiological experiment modeled by two-way profile analysis. This analysis is only exemplary, because the main aim of it was showing how theory works in practice. The analogous analysis was provided for variates other than dehydrogenase activity of soil. Results of those analysis will be published separately.

References

Casida L.E. Jr. (1977). Microbial metabolic activity in soil as measured by dehydrogenase determinations. *Appl. Environ. Microbiol*. 34, 630–636.

Zieliński W. (2009). Two-way profile analysis with interaction. *Colloquium Biometricum* (submitted).

Table. 1. Results for equal responses

Equal response of the fertlizer

Factor	t	w	F_{stat}	F_{crit}
F_1	3	50	115.5765	2.79
F_2	3	50	1.9603	2.79√
F_3	3	50	5.1829	2.79
F_4	3	50	52.3253	2.79
F_5	3	50	12.4063	2.79
F_6	3	50	12.5611	2.79
F_7	3	50	12.1669	2.79
F_8	3	50	3.0787	2.79
F_9	3	50	8.9734	2.79

Equal response of the year

Factor	t	w	F_{stat}	F_{crit}
Y_1	3	50	41.5570	2.79
Y_2	3	50	11.4873	2.79
Y_3	3	50	11.5738	2.79

Equal response of interactions

Factor	t	w	F_{stat}	F_{crit}
$F_1 \times Y_1$	3	50	205.295	2.79
$F_2 \times Y_1$	3	50	14.1350	2.79
$F_3 \times Y_1$	3	50	8.7035	2.79
$F_4 \times Y_1$	3	50	10.8833	2.79
$F_5 \times Y_1$	3	50	5.0391	2.79
$F_6 \times Y_1$	3	50	3.9339	2.79
$F_7 \times Y_1$	3	50	4.3760	2.79
$F_8 \times Y_1$	3	50	2.1301	2.79√
$F_9 \times Y_1$	3	50	4.0204	2.79
$F_1 \times Y_2$	3	50	6.9635	2.79
$F_2 \times Y_2$	3	50	3.6251	2.79
$F_3 \times Y_2$	3	50	3.3237	2.79
$F_4 \times Y_2$	3	50	64.6483	2.79
$F_5 \times Y_2$	3	50	1.9998	2.79√
$F_6 \times Y_2$	3	50	17.0995	2.79
$F_7 \times Y_2$	3	50	10.1739	2.79
$F_8 \times Y_2$	3	50	3.1026	2.79
$F_9 \times Y_2$	3	50	2.7810	2.79√
$F_1 \times Y_3$	3	50	96.7143	2.79
$F_2 \times Y_3$	3	50	4.1544	2.79
$F_4 \times Y_3$	3	50	1.2738	2.79√
$F_4 \times Y_4$	3	50	42.9803	2.79
$F_5 \times Y_3$	3	50	4.2874	2.79
$F_6 \times Y_3$	3	50	3.3801	2.79
$F_7 \times Y_3$	3	50	5.9681	2.79
$F_8 \times Y_3$	3	50	6.5965	2.79
$F_9 \times Y_3$	3	50	10.3278	2.79

Appendix

In the table there are given estimates of effects of factors involved in the experiment. To obtain those estimates the following constrains were applied:

$$\left.\begin{array}{l}\sum_{i=1}^{k}\alpha_{ih}=0,\ \sum_{j=1}^{c}\tau_{jh}=0\\ \sum_{i=1}^{k}\eta_{ijh}=0,\ (j=1,\ldots,c)\\ \sum_{j=1}^{c}\eta_{ijh}=0,\ (i=1,\ldots,k-1)\end{array}\right\}\ \text{for } h=1,\ldots,p.$$

	p=1	p=2	p=3	p=4	p=5
mean	0.322	0.311	0.365	0.382	0.374
F_1	0.468	0.633	0.866	0.826	0.652
F_2	-0.196	-0.196	-0.248	-0.224	-0.240
F_3	-0.220	-0.220	-0.292	-0.316	-0.278
F_4	0.233	0.268	0.322	0.313	0.473
F_5	-0.161	-0.121	-0.230	-0.263	-0.231
F_6	-0.092	-0.224	-0.290	-0.297	-0.279
F_7	0.360	0.310	0.418	0.354	0.414
F_8	-0.174	-0.192	-0.223	-0.179	-0.245
F_9	-0.220	-0.256	-0.323	-0.214	-0.265
Y_1	0.009	0.040	0.106	0.081	0.034
Y_2	0.090	0.061	0.051	0.033	0.084
Y_3	-0.099	-0.101	-0.157	-0.114	-0.119
$F_1 \times Y_1$	-0.118	-0.002	0.157	0.252	-0.270
$F_2 \times Y_1$	0.002	-0.008	-0.132	-0.109	-0.005
$F_3 \times Y_1$	-0.022	-0.069	-0.121	-0.087	-0.013
$F_4 \times Y_1$	0.312	0.176	0.278	0.179	0.271
$F_5 \times Y_1$	0.000	-0.081	-0.091	-0.087	-0.013
$F_6 \times Y_1$	-0.165	-0.077	-0.120	-0.086	-0.044
$F_7 \times Y_1$	0.142	0.140	0.190	0.244	0.167
$F_8 \times Y_1$	-0.118	-0.066	-0.088	-0.161	-0.053
$F_9 \times Y_1$	-0.033	-0.014	-0.073	-0.146	-0.040
$F_1 \times Y_2$	-0.053	-0.040	-0.223	-0.218	-0.149
$F_2 \times Y_2$	-0.123	-0.060	-0.030	0.016	-0.082
$F_3 \times Y_2$	-0.069	-0.022	0.001	0.025	-0.063
$F_4 \times Y_2$	-0.004	0.066	0.111	0.214	0.331
$F_5 \times Y_2$	-0.095	-0.095	-0.054	-0.022	-0.103
$F_6 \times Y_2$	0.181	-0.023	-0.058	-0.074	-0.123
$F_7 \times Y_2$	0.298	0.234	0.286	0.249	0.351
$F_8 \times Y_2$	-0.016	0.004	0.013	-0.063	-0.044
$F_9 \times Y_2$	-0.118	-0.063	-0.045	-0.127	-0.117
$F_1 \times Y_3$	0.172	0.042	0.066	-0.034	0.418
$F_2 \times Y_3$	0.121	0.068	0.162	0.092	0.088
$F_3 \times Y_3$	0.091	0.090	0.119	0.062	0.077
$F_4 \times Y_3$	-0.308	-0.242	-0.389	-0.392	-0.602
$F_5 \times Y_3$	0.095	0.176	0.145	0.109	0.117
$F_6 \times Y_3$	-0.015	0.100	0.179	0.160	0.168
$F_7 \times Y_3$	-0.440	-0.374	-0.476	-0.494	-0.519
$F_8 \times Y_3$	0.134	0.062	0.075	0.225	0.097
$F_9 \times Y_3$	0.150	0.077	0.118	0.273	0.157

DWUCZYNNIKOWA ANALIZA PROFILOWA Z INTERAKCJAMI: PRZYKŁAD ZASTOSOWANIA DO ANALIZY PEWNEGO EKSPERYMENTU MIKROBIOLOGICZNEGO

Streszczenie

W pracy pokazano zastosowanie modelu dwuczynnikowej analizy profilowej do wyników pewnego eksperymentu mikrobiologicznego. Podane są wartości statystyk testowych hipotez weryfikowanych wtakim modelu. Wyniki te zostały również zilustrowane na wykresach.

Słowa kluczowe: analiza profilowa, wielowymiarowa analiza statystycz\-na, doświadczenie dwuczynnikowe, doświadczenia mikrobiologiczne

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