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A STUDY ON THE INFLUENCE OF PINE EXTRACT UPON THE AIR MICROFLORA FROM DIFFERENT SPACES, USING THE MATHEMATICAL MODELISATION OF FACTORIAL TYPE

Anca Grădinaru (Ciobanu), Dumitra Răducanu, Ileana Denisa Nistor, Neculai Doru Miron, Gheorghe Brabie

Key words: *the air microbiota, pine extract, mathematical modeling, the factorial program*

INTRODUCTION

As we know, the effects of biological agents on the state of health of the persons from work environments with a rich microflora are harmful: professional illnesses such as allergies, asthma, allergic apergiloze, lung diseases, infections and also other types of toxic effects due to exposure at fungi, irritating effects of exposure to molds, immunological dysfunction, and eczemas. The work environment represented in our experiment from industrial hall is characterized by a heterogeneous microflora influenced by different physical-chemical factors. Knowledge of air microflora has a value sanitary epidemiological and is an indicator of pollution degree of air, of hygienic conditions in the various enclosures.

MATERIAL AND METHODS

The principle regarding the method of deposition of air consists in opening and exposure of Petri boxes with solid medium for several minutes and then their incubation in optimum conditions and counting of colonies formed.

Calculation of number total of germs (NTG) is achieved according to Omeleanski's formula:

$$Number\ of\ germs / m^3\ air = \frac{N \cdot 10000}{S \cdot T/5}$$

where: N – number of colonies formed
S – surface of Petri box
T – time of exposure

The culture medium used is a typical one of nutritive medium (composition meat extract 5g, Na Ca 2,5g, peptone 10g, agar 20 g, distilled water 1000ml).

The extract of pine used in the experiment was obtained by Soxhlet method. The solid material (needles) was placed inside a thimble made from thick filter paper, which was loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent (ethylic alcohol).

The solvent was heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material.

The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask.

This cycle was allowed to repeat ten times.After extraction the solvent was removed, typically by means of a rotary evaporator, yielding the extracted compound.

The non-soluble portion of the extracted solid was remained in the thimble, and was discarded. For this experiment we varied the amount of extract, the pH of environment of medium and time of exposure of Petri boxes, according to data presented in table 1.

Table 1. Parameters considered and their variation field

Parameter	Reduced variable	Minimum value	Average value	Maximum value	ΔX
		(-1)	(0)	(+1)	
Amount of extract (mL)	x_1	0	2.5	5	2.5
pH of environment of medium	x_2	6	7.5	9	1.5
Time of exposure (min.)	x_3	5	10	15	5

The response function studied was the total number of germs (NTG). Between brackets are notated the reduced values of variables. The results obtained are shown in table 2.

Table 2. Values of response functions obtained

No. crt.	Amount of extract (mL)	pH of environment of agar	Time of exposure (min.)	NTG (hall)	NTG (W.C.)	NTG (courtyard)
	x_1	x_2	x_3	Y_1	Y_2	Y_3
1	- 1 (0)	- 1 (6)	- 1 (5)	8649.84	0	4246.2845
2			0 (10)	0	4560.824	4324.92
3			+ 1 (15)	157.27	314.54	9855.5739
4		0 (7.5)	- 1 (5)	1887.24	314.54	7077.14
5			0 (10)	393.175	1022.25	8492.569
6			+ 1 (15)	209.693	157.27	1310.58
7		+ 1 (9)	- 1 (5)	471.81	2044.50	1729.97
8			0 (10)	707.714	1651.33	5111.27
9			+ 1 (15)	52.42	524.23	681.50
10	0 (2.5)	- 1 (6)	- 1 (5)	471.81	471.81	786.349
11			0 (10)	78.635	864.984	1415.43
12			+1 (15)	0	524.23	1363.0049
13		0 (7.5)	- 1 (5)	314.54	943.62	1572.698
14			0 (10)	78.635	157.27	1572.698
15			+ 1 (15)	157.27	209.693	3774.475
16		+ 1 (9)	- 1 (5)	471.81	157.27	7863.49
17			0 (10)	0	235.90	1258.16
18			+ 1 (15)	2568.74	209.693	7444.10
19	+1 (5)	-1 (6)	- 1 (5)	1887.24	471.81	629.08
20			0 (10)	864.984	0	2359.047
21			+ 1 (15)	524.23	157.27	943.62
22		0 (7.5)	- 1 (5)	786.349	471.81	1572.698
23			0 (10)	157.27	157.27	629.08
24			+ 1 (15)	262.11	262.11	1153.31
25		+1 (9)	- 1 (5)	1415.43	1415.43	2201.78
26			0 (10)	1022.25	235.90	1100.89
27			+ 1 (15)	314.54	314.54	786.349

For calculation of the significance regarding program effectuation was carried out three approval samples in the central point of the field (0, 0, 0), obtaining the values presented in table 3.

Table 3. Values in central point of the field

y_k^0	y_1^0	y_2^0	y_3^0
Value for NTG (hall)	157.27	157.27	471.81
Value for NTG (W.C.)	786.349	1100.89	471.81
Value for NTG (courtyard)	7548.95	471.81	1310.58

For we could elaborate the model of the response functions it was determinate the coefficients of the bottom polynomial for each response function (Azzouz A.).

$$Y = a_0 + a_1 \cdot x_1 + a_2 \cdot x_2 + a_3 \cdot x_3 + a_{11} \cdot x_1^2 + a_{22} \cdot x_2^2 + a_{33} \cdot x_3^2 +$$
$$+ a_{12} \cdot x_1 x_2 + a_{13} \cdot x_1 x_3 + a_{23} \cdot x_2 x_3 + a_{123} \cdot x_1 x_2 x_3$$

Values of coefficients of response function were calculated with the following relations:

$$y_0 = \frac{\sum_{k=1}^{27} Y_k}{27}$$

$$a_1 = \frac{\sum_{k=1}^{27} x_{1k} \cdot Y_k}{\sum_{k=1}^{27} x_{1k}^2}$$

$$a_2 = \frac{\sum_{k=1}^{27} x_{2k} \cdot Y_k}{\sum_{k=1}^{27} x_{2k}^2}$$

$$a_3 = \frac{\sum_{k=1}^{27} x_{3k} \cdot Y_k}{\sum_{k=1}^{27} x_{3k}^2}$$

$$a_{12} = \frac{\sum_{k=1}^{27} \left(x_1 \cdot x_2\right)_k \cdot Y_k}{\sum_{k=1}^{27} \left(x_1 \cdot x_2\right)_k^2}$$

$$a_{13} = \frac{\sum_{k=1}^{27} \left(x_1 \cdot x_3\right)_k \cdot Y_k}{\sum_{k=1}^{27} \left(x_1 \cdot x_3\right)_k^2}$$

$$a_{23} = \frac{\sum_{k=1}^{27} \left(x_2 \cdot x_3\right)_k \cdot Y_k}{\sum_{k=1}^{27} \left(x_2 \cdot x_3\right)_k^2}$$

$$a_{123} = \frac{\sum_{k=1}^{27} \left(x_1 \cdot x_2 \cdot x_3\right)_k \cdot Y_k}{\sum_{k=1}^{27} \left(x_1 \cdot x_2 \cdot x_3\right)_k^2}$$

$$a_{11} = \frac{\sum_{k=1}^{27} \left(x_1^2 - 2/3\right)_k \cdot Y_k}{\sum_{k=1}^{27} \left(x_1^2 - 2/3\right)_k^2}$$

$$a_{22} = \frac{\sum_{k=1}^{27} \left(x_2^2 - 2/3\right)_k \cdot Y_k}{\sum_{k=1}^{27} \left(x_2^2 - 2/3\right)_k^2}$$

$$a_{33} = \frac{\sum_{k=1}^{27} \left(x_3^2 - 2/3\right)_k \cdot Y_k}{\sum_{k=1}^{27} \left(x_3^2 - 2/3\right)_k^2}$$

$$a_0 = y_0 - \frac{2}{3} \cdot \left(a_{11} + a_{22} + a_{33}\right)$$

Values of these coefficients are presented in table 4.

Table 4. Values of coefficients of the polynomials

Coefficients	Response function Y_1 (NTG hall)	Response function Y_2 (NTG W.C.)	Response function Y_3 (NTG courtyard)
a_0	- 471.809	495.108	2922
a_1	- 294.153	- 394.63	- 1747
a_2	- 311.628	- 32.037	125.233
a_3	- 672.766	- 200.956	- 20.388
a_{11}	637.816	362.594	5.825
a_{22}	620.342	375.70	- 11.649
a_{33}	777.612	- 489.283	136.882
a_{12}	587.578	166.008	321.776
a_{13}	633.447	- 21.844	- 26.212
a_{23}	908.67	- 218.43	- 781.981
a_{123}	- 976.383	131.057	615.974

The form of elaborated models will be:

$$Y_1 = -471.809 - 294.153 x_1 - 311.628 \cdot x_2 - 672.766 \cdot x_3 + 637.816 \cdot x_1^2 + 620.342 \cdot x_2^2 + 777.612 \cdot x_3^2 +$$
$$+587.578 \cdot x_1 \cdot x_2 + 633.447 \cdot x_1 \cdot x_3 + 908.67 \cdot x_2 \cdot x_3 - 976.383 \cdot x_1 \cdot x_2 \cdot x_3$$
$$Y_2 = 495.108 - 394.63 \cdot x_1 - 32.037 \cdot x_2 - 200.956 \cdot x_3 + 362.594 \cdot x_1^2 + 375.7 \cdot x_2^2 - 489.283 \cdot x_3^2 +$$
$$+166.008 \cdot x_1 \cdot x_2 - 21.844 \cdot x_1 \cdot x_3 - 218.43 \cdot x_2 \cdot x_3 + 131.057 \cdot x_1 \cdot x_2 \cdot x_3$$
$$Y_3 = 2922 - 1747 \cdot x_1 + 125.233 \cdot x_2 - 20.388 \cdot x_3 + 5.825 \cdot x_1^2 - 11.649 \cdot x_2^2 + 136.882 \cdot x_3^2 +$$
$$+321.776 \cdot x_1 \cdot x_2 - 26.212 \cdot x_1 \cdot x_3 - 781.981 \cdot x_2 \cdot x_3 + 615.974 \cdot x_1 \cdot x_2 \cdot x_3$$

RESULTS AND DISCUSSIONS

It will be calculate the average of three approval samples performed for response function (NTG) in the central point of the field (0, 0, 0):

$$y_{med1}^0 = \frac{\sum_{i=1}^{3} y_i^0}{3} = \frac{157.27 + 157.27 + 471.81}{3} = 262.116$$
$$y_{med2}^0 = \frac{\sum_{i=1}^{3} y_i^0}{3} = \frac{786.349 + 1100.89 + 471.81}{3} = 786.349$$
$$y_{med3}^0 = \frac{\sum_{i=1}^{3} y_i^0}{3} = \frac{7548.95 + 471.81 + 1310.58}{3} = 3110.446$$

It calculates the mean square error with the relation, knowing that the number of samples, n, is 3:

$$\varepsilon^2 = \sum_{i=1}^{n} \frac{\left(y_i^0 - y_{med}^0\right)^2}{n-1}$$

	ε^2
Value for NTG (hall)	$3.298 \cdot 10^4$
Value for NTG (W.C.)	$9.894 \cdot 10^4$
Value for NTG (courtyard)	$1.495 \cdot 10^7$

It calculates the error in execution of approval samples:

$$\varepsilon = \sqrt{\varepsilon^2}$$

	ε
Value for NTG (hall)	181.6
Value for NTG (W.C.)	314.54
Value for NTG (courtyard)	$3.867 \cdot 10^3$

Determination the significance of coefficients is achieved by following relation, knowing that the number of experiments, N, is 27:

$$S = \frac{\varepsilon}{\sqrt{N}}$$

	S
Value for NTG (hall)	34.949
Value for NTG (W.C.)	60.533
Value for NTG (courtyard)	744.141

Significance of coefficients will appreciate using the test t-student with the relation:

$$t_j = \left|a_j\right| / S$$

Values of the test t-student for each coefficient are presented in table 5:

Table 5. Values of the test t-student

t_j	t_0	t_1	t_2	t_3	t_{12}	t_{13}	t_{23}	t_{11}	t_{22}	t_{33}	t_{123}
NTG (hall)	13.5	8.4	8.9	19.25	16.8	18.125	26	18.25	17.75	22.25	27.94
NTG (W.C.)	8.18	6.52	0.53	3.32	2.74	0.36	3.61	5.99	6.2	8.1	2.165
NTG (courtyard)	3.93	2.35	0.17	0.027	1.24	0.035	1.05	$7.83 \cdot 10^{-3}$	0.016	0.184	0.828

From results of test t-student we observe that can be eliminated following terms:

Terms eliminated for NTG (hall)	x_1, x_2, x_{123}
Terms eliminated for NTG (W.C.)	x_2, x_{13}, x_{123}
Terms eliminated for NTG (courtyard)	x_{11}, x_{22}, x_{123}

Mathematical model describing the response function of criterion optimization, after removing insignificant terms using the test t-student, is:

$$Y_1 = -471.809 - 672.766 \cdot x_3 + 637.816 \cdot x_1^2 + 620.342 \cdot x_2^2 + 777.612 \cdot x_3^2 + 587.578 \cdot x_1 \cdot x_2 +$$
$$+633.447 \cdot x_1 \cdot x_3 + 908.67 \cdot x_2 \cdot x_3$$
$$Y_2 = 495.108 - 394.63 \cdot x_1 - 200.956 \cdot x_3 + 362.594 \cdot x_1^2 + 375.7 \cdot x_2^2 - 489.283 \cdot x_3^2 +$$
$$+166.008 \cdot x_1 \cdot x_2 - 218.43 \cdot x_2 \cdot x_3$$
$$Y_3 = 2922 - 1747 \cdot x_1 + 125.233 \cdot x_2 - 20.388 \cdot x_3 + 136.882 \cdot x_3^2 + 321.776 \cdot x_1 \cdot x_2 -$$
$$-26.212 \cdot x_1 \cdot x_3 - 781.981 \cdot x_2 \cdot x_3$$

Forwards will be discussing the effects of parameters.

- The value of a_0 is negative (- 471.809) which indicates that we have an insignificant number of germs. This result may be the effect of factors with bactericidal or germicidal action (physical-chemical factors), fungi static or fungicides.
- If the coefficients a_1, a_2 and a_3 are positives, follow that the variables x_1, x_2 and x_3 have a favorable action, but if the coefficients a_1, a_2 and a_3 are negatives, follow that the variables x_1, x_2 and x_3 have a individual action unfavorable for the existence of microorganisms.
- If the interaction coefficients a_{12}, a_{13} and a_{23} are positives follow that variables x_1 and x_2, x_1 and x_3, and x_2 and x_3 by their interaction, have a favorable effect of presence of microorganisms, with the same intensity as their interaction, and if these coefficients are negative it follow that the variables x_1 and x_2, x_1 and x_3, and x_2 and x_3 by their interaction, have an unfavorable effect of presence of microorganisms.
- Analyzing the quadratic coefficients a_{11}, a_{22} and a_{33} follows that the response function Y_1 (from table 4) is characterized by a minimum in proportion to the variables x_1, x_2 and x_3, the response function Y_2 (from table 4) is characterized by a minimum in proportion to the variables x_1 and x_2 and by a maximum in proportion to the variable x_3, and the response function Y_3 (from table 4) is characterized by a maximum in proportion to variable x_1 and by a minimum in proportion to variables x_2 and x_3.

The elaborated models provide information on the individual's effects and interactions and additional data with the aid of quadratic terms in the sense that their values and signs give a clear idea about areas response concavity or convexity.

For the response function obtained after removing of insignificant terms using test t-student will calculate the partial derivatives of first order in proportion to each variable. Partial derivatives of first order obtained are equate to 0 and it solves the linear system resulted. So we obtain:

$$For\ Y_1 \Rightarrow \begin{cases} x_1 = -0.16 \\ x_2 = -0.50 \\ x_3 = 0.786 \end{cases} \quad For\ Y_2 \Rightarrow \begin{cases} x_1 = 0.60 \\ x_2 = -0.20 \\ x_3 = -0.16 \end{cases} \quad For\ Y_{31} \Rightarrow \begin{cases} x_1 = 0.46 \\ x_2 = 0.25 \\ x_3 = 0.70 \end{cases}$$

The optimal point request is (-0.16; -0.50; 0.786) for Y_1, (0.60; -0.20; -0.16) for Y_2 and (0.46; 0.25; 0.70) for Y_3, in adimensional coordinates. As we can see the optimum values for x_1, x_2 and x_3 are within the limits of allowable domain settled initial (-1, 1).

Knowing the fields of variation of amount of extract, pH of environment of medium and time of exposure we can obtain the real values of the optimal point using the following relations:

$$X_1 = \Delta X_1 \cdot x_1 + X_1^{med}$$
$$X_2 = \Delta X_2 \cdot x_2 + X_2^{med}$$
$$X_3 = \Delta X_3 \cdot x_3 + X_3^{med}$$

where:
X_1, X_2, X_3 – real values of optimum
x_1, x_2, x_3 – adimensionale values of optimum
ΔX_1, ΔX_2, ΔX_3 – step of each variation domain
X_1^{med}, X_2^{med}, X_3^{med} – real average value of parameters

$$X_1 = \Delta X_1 \cdot x_1 + X_1^{med}$$
$$X_2 = \Delta X_2 \cdot x_2 + X_2^{med}$$
$$X_3 = \Delta X_3 \cdot x_3 + X_3^{med}$$

where:
X_1, X_2, X_3 – real values of optimum
x_1, x_2, x_3 – adimensionale values of optimum
ΔX_1, ΔX_2, ΔX_3 – step of each variation domain
X_1^{med}, X_2^{med}, X_3^{med} – real average value of parameters

$$For\ Y_1 \Rightarrow \begin{cases} X_1 = 2.5 \cdot (-0.16) + 2.5 = 2.1\ mL \\ X_2 = 1.5 \cdot (-0.50) + 7.5 = 6.75 \\ X_3 = 5 \cdot 0.786 + 10 = 13.93\ minutes \end{cases}$$

$$For\ Y_2 \Rightarrow \begin{cases} X_1 = 2.5 \cdot 0.6 + 2.5 = 4.00\ mL \\ X_2 = 1.5 \cdot (-0.20) + 7.5 = 7.20 \\ X_3 = 5 \cdot (-0.16) + 10 = 9.20\ minutes \end{cases}$$

$$For\ Y_3 \Rightarrow \begin{cases} X_1 = 2.5 \cdot 0.46 + 2.5 = 3.65\ mL \\ X_2 = 1.5 \cdot 0.25 + 7.5 = 7.87 \\ X_3 = 5 \cdot 0.70 + 10 = 13.50\ minutes \end{cases}$$

The obtained models will be represented graphically in function of two parameters, the other parameter remaining constant at a value of 0 which is the domain of variation (fig. 1 to 9).

For Y_1 (NTG from hall) will be three areas of response characterized by the following mathematical models:

$x_1 = 0$	$Y_1 = -471.809 - 672.766 \cdot x_3 + 620.342 \cdot x_2^2 + 777.612 \cdot x_3^2 + 908.67 \cdot x_2 \cdot x_3$
$x_2 = 0$	$Y_1 = -471.809 - 672.766 \cdot x_3 + 637.816 \cdot x_1^2 + 777.612 \cdot x_3^2 + 633.447 \cdot x_1 \cdot x_3$
$x_3 = 0$	$Y_1 = -471.809 + 637.816 \cdot x_1^2 + 620.342 \cdot x_2^2 + 587.578 \cdot x_1 \cdot x_2$

The graphs charts of these mathematical models are represented in the fig. 1 to 3:

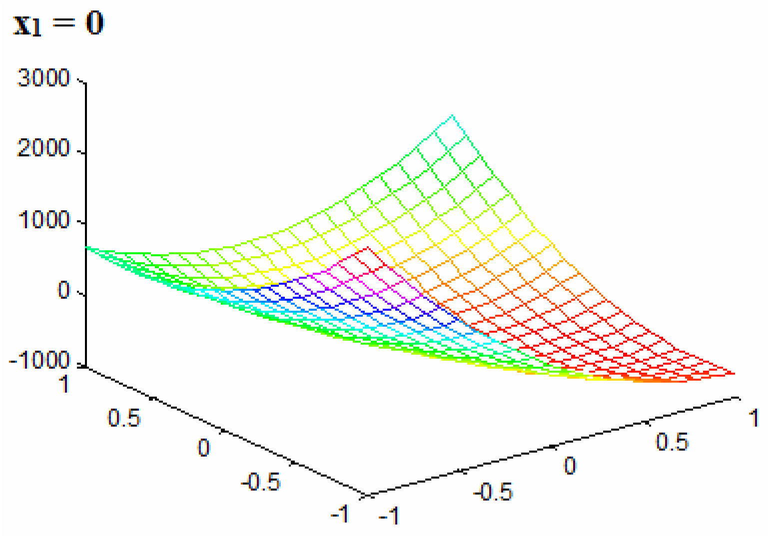

Fig. 1. Influence of pH of environment of agar and time of exposure on total number of germs when the amount of extract is hold in centered domain

$$Y_1 = -471.809 - 672.766 \cdot x_3 + 620.342 \cdot x_2^2 + 777.612 \cdot x_3^2 + 908.67 \cdot x_2 \cdot x_3$$

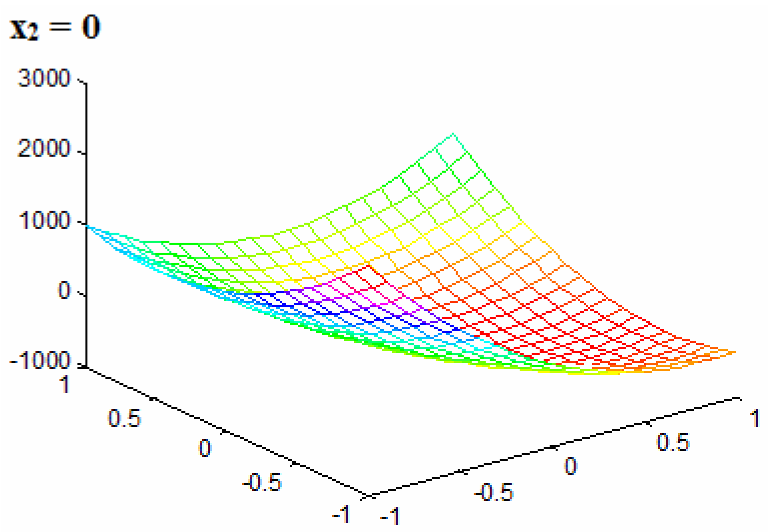

Fig. 2. Influence of amount of extract and time of exposure on total number of germs when pH of environment of medium is hold in centered domain

$$Y_1 = -471.809 - 672.766 \cdot x_3 + 637.816 \cdot x_1^2 + 777.612 \cdot x_3^2 + 633.447 \cdot x_1 \cdot x_3$$

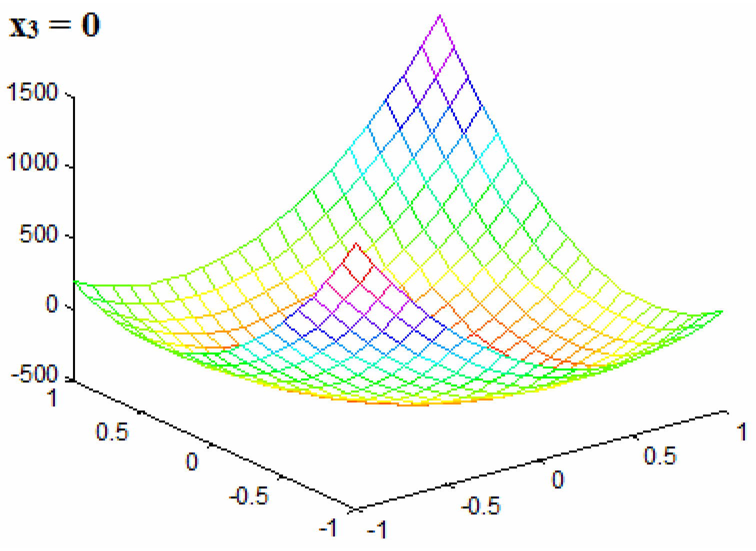

Fig. 3. Influence of amount of extract and pH of environment of agar on total number of germs when time of exposure is hold in centered domain

$$Y_1 = -471.809 + 637.816 \cdot x_1^2 + 620.342 \cdot x_2^2 + 587.578 \cdot x_1 \cdot x_2$$

- As long as both coefficients of quadratic terms have positive signs, then the quadratic terms determines a minimum and response surfaces (fig. 1, 2 and 3) which correspond to the models are convex (crater or hole).

For Y_2 (NTG from toilets) will be three areas of response characterized by the following mathematical models:

$x_1 = 0$	$Y_2 = 495.108 - 200.956 \cdot x_3 + 375.7 \cdot x_2^2 - 489.283 \cdot x_3^2 - 218.43 \cdot x_2 \cdot x_3$
$x_2 = 0$	$Y_2 = 495.108 - 394.63 \cdot x_1 - 200.956 \cdot x_3 + 362.594 \cdot x_1^2 - 489.283 \cdot x_3^2$
$x_3 = 0$	$Y_2 = 495.108 - 394.63 \cdot x_1 + 362.594 \cdot x_1^2 + 375.7 \cdot x_2^2 + 166.008 \cdot x_1 \cdot x_2$

The graphs charts of these mathematical models are represented in the fig. 4 to 6:

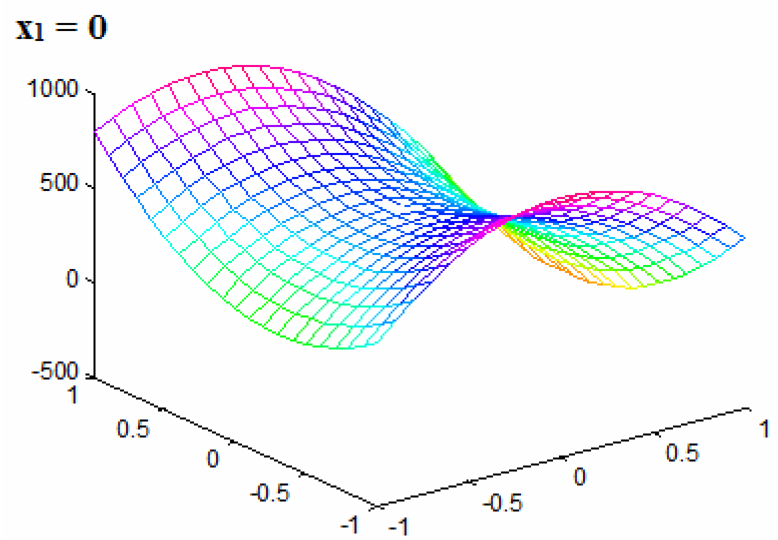

Fig. 4. Influence of pH of environment of medium and time of exposure on total number of germs when the amount of extract is hold in centered domain

$$Y_2 = 495.108 - 200.956 \cdot x_3 + 375.7 \cdot x_2^2 - 489.283 \cdot x_3^2 - 218.43 \cdot x_2 \cdot x_3$$

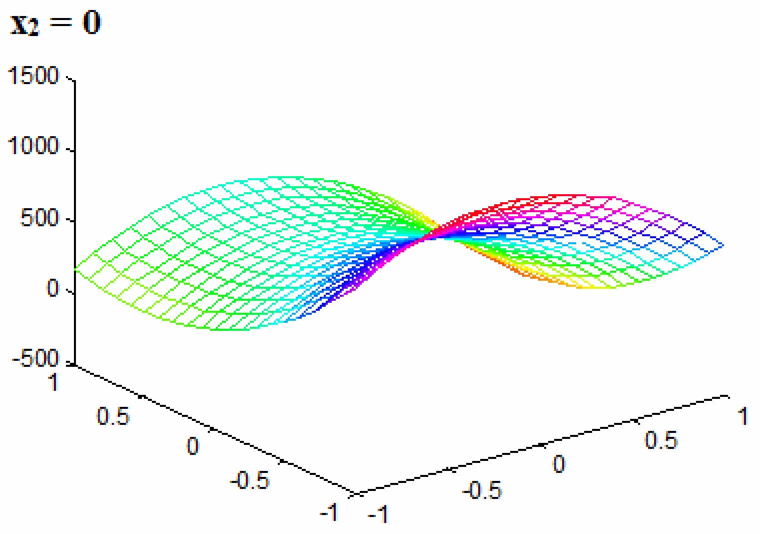

Fig. 5. Influence of amount of extract and time of exposure on total number of germs when pH of environment of medium is hold in centered domain

$$Y_2 = 495.108 - 394.63 \cdot x_1 - 200.956 \cdot x_3 + 362.594 \cdot x_1^2 - 489.283 \cdot x_3^2$$

- As long as the coefficients of quadratic terms have contrary signs, then the response surfaces (figures 4 and 5) which correspond to the models have horseback form.

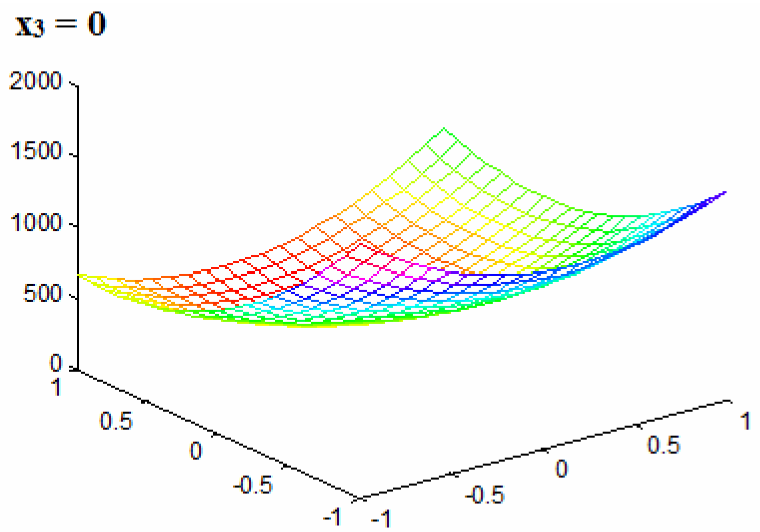

Fig. 6. Influence of amount of extract and pH of environment of agar on total number of germs when time of exposure is hold in centered domain

$$Y_2 = 495.108 - 394.63 \cdot x_1 + 362.594 \cdot x_1^2 + 375.7 \cdot x_2^2 + 166.008 \cdot x_1 \cdot x_2$$

- As long as both coefficients of quadratic terms have positive signs, then the quadratic terms determines a minimum and response surface (figure 6) which correspond to the models is convex (crater or hole).

For Y_3 (NTG from courtyard) will be three areas of response characterized by the following mathematical models:

$x_1 = 0$	$Y_3 = 2922 + 125.233 \cdot x_2 - 20.388 \cdot x_3 + 136.882 \cdot x_3^2 - 781.981 \cdot x_2 \cdot x_3$
$x_2 = 0$	$Y_3 = 2922 - 1747 \cdot x_1 - 20.388 \cdot x_3 + 136.882 \cdot x_3^2 - 26.212 \cdot x_1 \cdot x_3$
$x_3 = 0$	$Y_3 = 2922 - 1747 \cdot x_1 + 125.233 \cdot x_2 + 321.776 \cdot x_1 \cdot x_2$

The graphs charts of these mathematical models are represented in the figures 7 to 9:

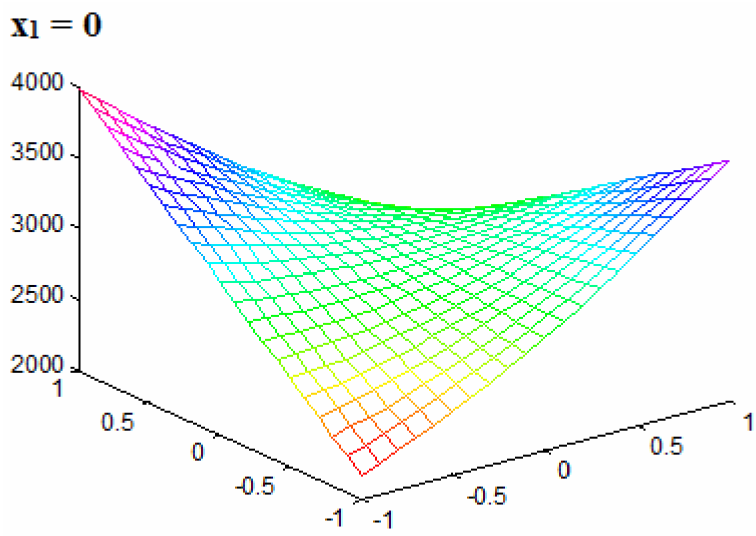

Fig. 7. Influence of pH of environment of medium and time of exposure on total number of germs when the amount of extract is hold in centered domain

$$Y_3 = 2922 + 125.233 \cdot x_2 - 20.388 \cdot x_3 + 136.882 \cdot x_3^2 - 781.981 \cdot x_2 \cdot x_3$$

- As long as the coefficient of quadratic term has positive sign, then the response surface (figure 7) which corresponds to the model present a minimum in proportion to variable x_3.

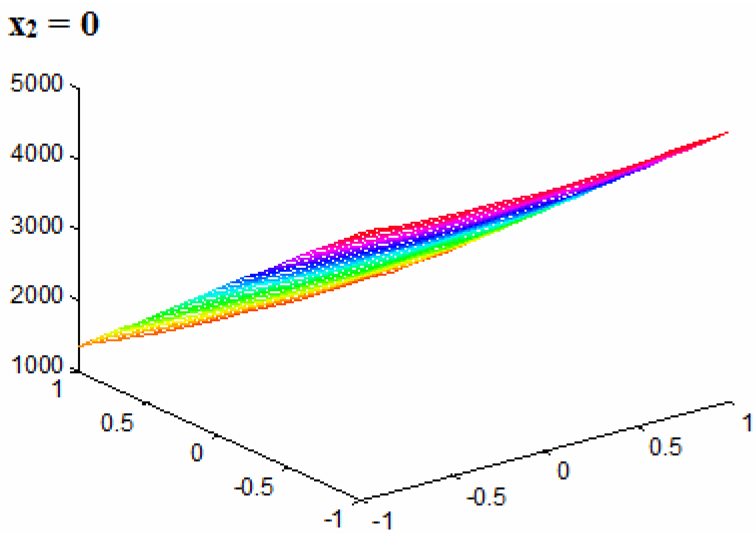

Fig. 8. Influence of amount of extract and time of exposure on total number of germs when pH of environment of medium is hold in centered domain

$$Y_3 = 2922 - 1747 \cdot x_1 - 20.388 \cdot x_3 + 136.882 \cdot x_3^2 - 26.212 \cdot x_1 \cdot x_3$$

- As long as the coefficient of quadratic term has positive sign, then the response surface (figure 8) which corresponds to the model present a minimum in proportion to variable x_3.

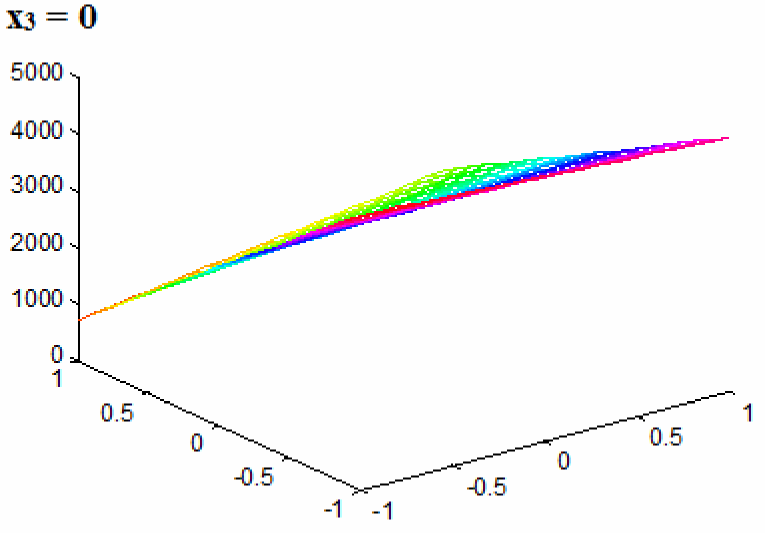

Fig. 9. Influence of amount of extract and pH of environment of medium on total number of germs when time of exposure is hold in centered domain

$$Y_3 = 2922 - 1747 \cdot x_1 + 125.233 \cdot x_2 + 321.776 \cdot x_1 \cdot x_2$$

CONCLUSIONS

From graphic representations, there can be distinguished roundness areas, the minimum and maximum due of quadratic coefficients effects, and also the inflexion point.

From the real values of optimum resulted after the calculation we can detach the following observations:

- The optimum amount of extract of pine is 2.1 mL for Y_1, 4 mL for Y_2 and 3.65 mL for Y_3;
- The optimum pH of environment for cultural medium is 6.75 for Y_1, 7.20 for Y_2 and 7.87 for Y_3;
- The optimum time of exposure is 13.93 minutes for Y_1, 9.20 minutes for Y_2 and 13.50 minutes for Y_3.

In conclusion, we can say that the total number of germs tends to optimum value when all considerate variables remain within the limits of variation domain initially chosen (amount of extract: 0 – 5 mL, pH of environment of agar: 6 – 9, time of exposure: 5 – 15 minutes).

Analyzing the total number of germs and the influence of physical-chemical factors of three selected environments (hall, W.C. and courtyard) it can observe that the highest number of microorganisms was in the courtyard, absolutely normal because of favorable conditions to development of microorganisms (temperature, humidity, light, wind, absence of chemical substances), aspect that is not reflected in the other two spaces.(fig10. a,b).

The low total number of germs from hall is due on the one hand to presence of bactericide substances (acids, ethers, alcohols, metal compounds, oxides) and on the other hand to unfavorable physical factors (temperature, excessive humidity, radiations).

The abundant presence of microorganisms in toilets reflects a faulty disinfection of baths and a favorable environment to the development of microorganisms, so it is necessary a better hygiene of baths.

ABSTRACT

In this paper, there is tested the influence of pine extract (needles) on the microbiota air from different enclosures: industrial hall, toilet and university yard. The achievement of this study is necessary because the complex microbiota (bacteria, yeasts, molds spores, fungi) in the air have epidemediologically regulated standards (quantitative and qualitative). The microbiological analysis of the air, in the three spaces, is made according to the Koch method (method of air sedimentation). The calculation of NTG (the total number of germs) is achieved using the formula of Omeleanski.

The experiment was conducted during spring time (15-22.03.2009), using the factorial program of type 3^3. The mathematical modeling of factorial type 3^3 involves a set of 27 experiments and three additional experiments used as approval samples. (Nistor I.D.).

We considered three levels of variation, as follows: amount of extract (ml);pH of environment of culture medium; time of exposure (minutes).

Fig 10 a. Preparation of culture medium with pine extract

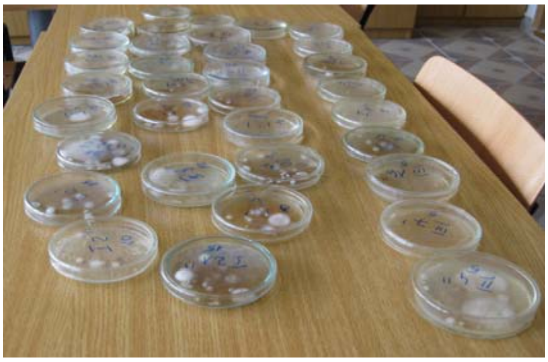

Fig 10 b. The Petri's with colonies

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AUTHOR'S ADDRESS

GRĂDINARU (CIOBANU) ANCA, NISTOR ILEANA DENISA, MIRON NECULAI, BRABIE GHEORGHE - University of Bacau, Faculty of Engineering at the Department of Engineering Chemistry, Calea Mărăşeşti Street, No 157, 600115, Bacău, Romania,
e-mail: ancutica2220@yahoo.com

RĂDUCANU DUMITRA - The Bacău University, Faculty of Sciences, Department of Biology, Calea Mărăşeşti Street, No 157, 600115, Bacău, Romania,
e-mail: dumitra_manea@yahoo.com.