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MODELLING A SYSTEM FOR „DISINFECTION-UTILIZATION“ OF SLUDGES FROM A PURIFICATION PLANT FOR WASTE WATERS

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ABSTRACT

Sludge from a purification plant for waste waters are studied for presence of pathogenic and non-pathogenic microflora. Their disinfection is carried out by applying lime treatment (10%, 20%, 30% lime) with two species vegetation – *lavender* (*Lavandula*) and *basil* (*Ocimum*). The results obtained after the processing of the sludge about the quantity microflora in them are studied for presence of a correlation connection between the concentration of the lime solution and the quantity of microorganisms. The obtained results show a strong correlation connection among the studied quantities. This gives grounds to be looked for a mathematical model on description of the microflora quantity in the sludge. More factors are added for the purpose – temperature, humidity and pH. In the article are presented the regression equations for the different methods of disinfection. The results give grounds to be affirmed that most of them can be used successfully for finding the quantity microflora in studied sludge with known data for concentration of the solution, temperature, humidity and pH.

Keywords: model, sludge, microorganisms, lime treatment, correlation, regression analysis

INTRODUCTION

Time dictates the necessity of speeding up the elaboration of projects for new construction and reconstruction of waste waters equipment. This in turn sets a number of other tasks for perfection of the used technologies, for equipment of new generation, complying with the best world standards with increased efficiency. The automation of the processes with purpose energy saving and stabilization of the sludge quality, which can be used in agriculture, is not less important. The mechanism of interrelated complicated and numerous technological calculations of the purification equipment for waste waters, including the systems for sludge purification and their subsequent treatment by the help of instruments for mathematical modelling, is not sufficiently studied until the moment.

The sludge from purification plants is organic substrate, which is appropriate for fertilization in agriculture after disinfection. Useful microorganisms are isolated in them (non-spore forming bacteria, bacilli, actinomycetes, micromycetes, bacteria, assimilating mineral nitrogen), participating in the decomposition of organic substances (**Malcheva et al., 2021, Malcheva et al., 2022**), as well as pathogenic microflora like: *Salmonella sp*., *Listeria sp*., *Escherichia coli*, *Campylobacter sp*., *Clostridium sp*., *Yersinia sp*., *Enterococcus* and others (**Malcheva et al., 2021, Malcheva et al., 2022, Arthurson et al., 2008, Dudley et al., 1980, Larsen et al., 1986, Straub et al., 1993, Strauch et al., 1991, Dermendzhieva, 2017**). The lime treatment is one of the disinfection methods of sludge from pathogens (**Malcheva et al., 2021, Malcheva et al., 2022, Amer et al., 1997, Jepsen et al., 1997, Wong et al., 2000, Marinova et al., 2016, Popova et al., 2014**). Some plants synthesize flavonoids as a response of a microbial infection and it is established that these compounds are a powerful antimicrobial agent against a wide spectrum of pathogenic microorganisms (**Górniak, et al., 2019**).

The microorganisms are sensitive indicators, some of them like *Clostridium* are spore forming and their destruction is more difficult. The study of microbes in dynamics is necessary for providing complete disinfection of pathogens on the one hand, on the other hand for following the development of the beneficial microorganisms after treatment.

Regardless of the sludge treatment method for disinfection of pathogenic microorganisms (lime treatment, application of formaldehyde) some authors for statistic processing of the results use classic methods, as classic method of Student-Fisher (**Popova et al., 2014, Popova, Baykov et al., 2014**). One of the most widespread models for disinfection of sludge is the one suggested by Chick-Watson, in which is included the initial concentration of the microorganisms, the concentration of remaining microorganisms at moment t, pseudo constant of speed of reaction from first line, the concentration of the disinfectant and empirical coefficient, often accepted for equal to 1 (**Pernitsky et al., 1995, Merdez et al.,2004**). In 1972 Hom (**American Water Works, 1990**) suggested an alternative kinetic model for reporting diversions from the Chick-Watson's model, often met in practice. The model introduces an empirical constant. Some authors present kinetic coefficients for creation of a modified Hom's model, obtained with disinfection of sludge by ammonia (**Merdez et al.,2004**). About solving this type of tasks and presenting the results reliably can be used important mathematical conceptions, to be created mathematical models, which shall assist modelling a system „disinfection-utilization of sludge" from purification plants for application in the agricultural practice.

The purpose of the scientific development is via the mathematical usage to be created a model of a sustainable system, applicable with sludge disinfection from purification plants for waste waters of pathogenic microorganisms, by lime treatment and phytoremediation. In the process of disinfection is followed the biogenicity of the beneficial microflora after treatment. The model assists the safety assessment and the sludge quality for its application as an organic fertilizer in agriculture.

MATERIALS AND METHODS

Sludge from a purification plant have been analyzed as per microbiological indicators (pathogenic and non-pathogenic microflora), in dynamics for a period of 40 days, composted in greenhouse conditions, in plastic containers, treated with quicklime in different concentrations (10%, 20%, 30%) and variants – without vegetation and with vegetation - *lavender (Lavandula)* and *basil (Ocimum)* (table 1).

Used experimental methods

The microbiological analyses are carried out as per the method of the limit dilutions, culture on solid nutrient media, subsequent cultivation (in a thermostat for aerobes and in a jar with reagent for anaerobes) and results reporting (cfu/gr. substrate). For isolation of the microorganisms are used the following nutrient media: non-pathogenic microflora: Nutrient agar for non-spore-forming bacteria and bacilli; MRS agar for lactobacilli; Actinomycetes isolation agar for actinomycetes and bacteria, assimilating mineral nitrogen; Czapek-Dox agar for moulded fungi; pathogenic microflora: Desoxycholate citrate agar for Salmonella

sp.; Endo agar for *Escherichia coli and coliforms* (oxidase confirming test); ChromoBio *Enterococcus* agar - for *Enterococcus*; Perfringens agar (TSC and Perfringens selective supplement) for *Clostridium perfringens*.
The analysis of the microbiological indicators is presented in detail in a previous scientific material (**Malcheva et al.,2021**).
For following the sludge temperature is used a temperature probe, for determining the moisture – a moisture meter, and for registering pH (in water) – pH meter.

Table 1 Scheme of the variants and period of microbiological analysis

Variants	Microbiological analysis
Without vegetation	Pathogenic and non-pathogenic microflora: Before starting the experiment; Day 1, 6 hours after starting of the experiment; 5th day after starting the experiment; 20th day after starting the experiment; 40th day after starting the experiment
S1 (0%) Sludge without quicklime (CaO)	
S2 (10%) Sludge +10% CaO	
S3 (20%) Sludge +20% CaO	
S4 (30%) Sludge +30% CaO	
With vegetation	
S5 (0%; L) Sludge without quicklime (CaO)+ Lavandula	
S6 (10%; L) Sludge +10% CaO + Lavandula	
S7 (20%; L) Sludge +20% CaO + Lavandula	
S8 (30%; L) Sludge +30% CaO + Lavandula	
S9 (0%; O) Sludge without quicklime (CaO)+ Ocimum	
S (10%; O) Sludge +10% CaO + Ocimum	
S (20%; O) Sludge +20% CaO + Ocimum	
S (30%; O) Sludge +30% CaO + Ocimum	

Figure 1 Vessel experience in a greenhouse.

Statistic processing of the data

With presence of great number of experimentally obtained data the most natural approach to their processing is the usage of the mathematical statistics methods. When carrying out experimental studies with scientific purpose, the main striving is often for finding mathematical formulations, which shall give formal description of the obtained dependencies. The mathematical statistics gives abundant instruments for discovering a connection between studied quantities. First step, before choosing a concrete algorithm is choosing the quantities, which are followed and formulation of the results, which are important for the study and about which mathematical descriptions are searched. Here is proceeded from a preliminarily group of quantities formation – factors, quantities, which are changed during the study and about which the researcher reckons that they affect the results from the experiments. The a priori information about the variable quantities is important, because thanks to it, it is often known whether there are statistic dependencies between the variables preliminarily. The selection of the next algorithms often depends on this. If the researcher does not have a priori information about this or is afraid of hidden connections between the variables, is appropriate to be carried out preliminary statistic checkups, which shall show the presence of dependencies between the variables. The correlation coefficient is usually used for description of the power and direction of dependency between variable quantities. It is a statistic measure about the dependency between two random variable quantities x and y.

$$\rho = \frac{Cov(x,y)}{\sqrt{Var(x).Var(y)}} \quad (1),$$

Where Cov(x,y) and Var(x),Var(y) are respectively the covariation and the variations of the quantities x and y.

If the random quantities are independent, the value of ρ is 0, with dependent quantities, it shall accept value 1. Pearson's coefficient is often used instead of the correlation coefficient. It is the most widespreadly used measure for straight-line dependency. It is usually marked with R and is changed within the boundaries from -1÷1. It is calculated as per the formula:

$$R_{yx} = \sqrt{1 - \frac{S_y^2}{\sigma_y^2}} \quad (2),$$

Where S_y^2 is the residual dispersion of the dependent variable, and σ_y^2 is the total dispersion of the dependent variable. There is not dependency with value 0, and with 1 or -1 it is said that a perfect linear connection between the variables is available. By the obtained values of the Pearson's coefficient can be drawn a conclusion about the dependency strength between the considered quantities.

- 0 < R < 0,3 – weak correlations
- 0,3 < R < 0,5 – moderate correlation
- 0,5 < R< 0,7 – significant correlation
- 0,7 < R< 0,9 – high correlation
- 0,9 < R < 1 – very high correlation

The Pearson's correlation coefficient can be applied for measuring the dependency strength of linear and non-linear single and multiple correlation connections.
A diagram of dispersion is often built in addition to the correlation coefficient (**Dowdy et al., 2004**). The practice has shown that often the determination coefficient is easier for work. It is the squared coefficient of R. It shows what percent of the changes in the factor variable shall lead to changes in the result variable.
One of the most frequently used means for finding a mathematical method, describing the dependency between one or more factors (independent variable quantities) and result from the study (dependent variable) is the regression analysis (**Krämer et al., 1983, Carlberg et al., 2016**). It gives opportunity for a profound analysis and modelling of correlation dependencies between occurrences. Its basic purpose is presenting in analytical type a studied correlation dependency, the so called mathematical model. The total type of the mathematical model is:

$$Y = f(X_j) + \varepsilon \quad (3),$$

where f(Xj) is the functional part of the model, and ε is a stochastic component. The functional part is also called determined.
This is one of the main reasons the method to be used so frequently – the result from it is analytical – mathematical description, a polynome. Another advantage of the regression analysis is its good recognition in the scientific circles and because of this its inclusion as a standard function in number of software products, thanks to which it can easily be applied and used even with presence of large amount of data. One of the software products, in which the procedure is available as a standard function is MS Excel (**Carlberg et al., 2016**).
Unfortunately, the regression analysis does not give an answer to the question what the reasons for the obtained results are. It only manifests whether there is a connection between the studied quantities. It is useful for finding the type of dependency, and what is more important, for quantitative determination of parameters of the selected function. The latter is very important if we are looking not only for analytical description of a certain experiment, and we want to reach further – to prognostication of future results from such subsequent experiments.
For carrying out a regression analysis are often used widely known software products, specialized like Matlab, as well as widely available like Excel. By their help each beginner can carry out the respective analysis. However, the interpretation of the obtained results is more important. Besides the mathematical dependency on the type (3) with carrying out a regression analysis are obtained and other numerical results, as per which values can be drawn great number of conclusions.
The task with creation of a regression model is firstly to be found the dependency $F(X_i)$. It is usually a polynome of the type: $\beta_0 + \beta_1 X_i + \beta_2 X_{i2} + \ldots$. Here $\beta_1, \beta_2, \ldots, \beta_\kappa$ are coefficients of the regression equation or parameters of the model. The reason for the second component, the stochastic, is that the correlation dependencies are not strictly determined and cannot be expressed analytically only by the indicated mathematical function. In fact, the stochastic component reflects the impact of other factors, which affect the resultant occurrence, but do not participate in explicit aspect in the regression model. These can be factors of accidental character or such which the researcher has not found and has not included with creation of the model in the concrete case. The values of the stochastic component appear residual, as a difference between the empirical and estimated values of the dependent variable.

RESULTS

The statistic processing of the data includes a preliminary correlation analysis for finding the connections between the obtained results for pathogenic and non-pathogenic microflora and the different conditions, with which the experiments are carried out – concentration of the lime solution, planting with different crops (lavender, basil). The purpose of this analysis is preliminary finding of the

connections between the considered quantities with purpose easier creation of a mathematical model at a later stage.

Correlation and dispersion analysis for determining the impact of the different concentration lime solution on the pathogenic microflora

Treatment only with lime solution

Table 2 Results for the coefficient of correlation between different concentration of lime solution and the presence of pathogenic microflora

Pathogenic microflora	Correlation coefficient
Coliforms and Escherichia coli	-0,65523
Enterococcus	-0,6777
Clostridium perfringens	-0,62592

With all the three types of pathogenic microflora the coefficients of correlation between the quantity of the indicated type of microflora and the concentration of the lime solution is less than -0.6. The conclusion is that between the solution for treatment concentration and the pathogenic microorganism's quantity in the sludge, exists a significant correlation or a negative linear connection. Consequently, when searching a mathematical model for description of the quantity pathogenic microflora in treated with lime solution sludge is appropriate to be used the percent of the lime solution as one of the variables.
The presence of a connection between the microorganism's quantity and the solution concentration, used for treatment of the sludge is also proven by a carried-out dispersion analysis. The value of F_{crit} is 3,49. For the separate types of pathogens is obtained:

Table 3 Value of the dispersion ratio with the different types of pathogenic microflora

Fcrit	F		
	***Escherichia coli* and coliforms**	***Enterococcus***	***Clostridium perfringens***
3,49	8,88	10,54	6,28

In all indicated cases $F>F_{crit}$

Planting lavender on sludge treated with lime solution

The results with the planted with *lavender* and treated by lime solution sludge, regarding dependency between solution concentration and quantity *Escherichia coli* and coliforms are similar to these, which are obtained from the sludge, which are treated only by lime solution, without planting *lavender* (Table 7).

Table 4 Results for the coefficient of correlation between different concentration of lime solution with planting *lavender* and the presence of pathogenic microflora

Pathogenic microflora	Correlation coefficient
Escherichia coli and coliforms	-0,65607
Enterococcus	-0,6777
Clostridium perfringens	-0,62592

The coefficients of correlation and in this case show a significant negative linear correlation. The same tendency is also noticed with the dispersion analysis:

Table 5 Value of the dispersion ratio with the different types of pathogenic microflora

Fcrit	F		
	***Escherichia coli* and coliforms**	***Enterococcus***	***Clostridium perfringens***
3,49	8,83	10,27	6,35

The planting of lavender on the sludge does not cancel the dependency between the concentration of the lime solution and the quantity of pathogenic microflora. A difference is observed between the quantity of pathogenic microorganisms in the cases without and with lavender, but inspite of this the concentration of the lime solution remains a significant factor.

Planting basil on sludge treated with lime solution

The planting of the sludge with basil regarding the statistic dependencies between the pathogenic microorganisms and the concentration of the lime solution has analogous results, like with the planting of lavender.

Table 6 Results for the coefficient of correlation between different concentration of lime solution with planting basil and the presence of pathogenic microflora

Pathogenic microflora	Correlation coefficient
Escherichia coli and coliforms	-0,65431
Enterococcus	-0,67667
Clostridium perfringens	-0,6289

The results from a dispersion analysis are shown in the next table (Table 7):

Table 7 Value of the dispersion ratio with the different types of pathogenic microflora

Fcrit	F		
	***Escherichia coli* and coliforms**	***Enterococcus***	***Clostridium perfringens***
3,49	8,68	10,52	6,35

The obtained data is very close to these with planting lavender.

Correlation and dispersion analysis for determining the impact of the different lime solution concentration on non-pathogenic microflora

The calculated correlation coefficients regarding the quantity of non-pathogenic microflora with respect to the concentration of the used lime solution and the planting with lavender and basil can be seen in table 11.

Table 8 Results for the correlation coefficient with experiments with non-pathogenic microflora

Treatment	Non-pathogenic microflora	Coefficient of correlation
Only with lime solution	Non-spore forming bacteria	-0,72615760
	Bacilli/Lactobacilli	-0,818387935
	Actinomycetes	-0,754731908
	Micromycetes	-0,93773642
	Bacteria, assimilating mineral nitrogen	-0,68576471
Lime solution in different concentrations and planting with lavender	Non-spore forming bacteria	-0,730202331
	Bacilli/Lactobacilli	-0,741122278
	Actinomycetes	-0,67909975
	Micromycetes	-0,920521853
	Bacteria, assimilating mineral nitrogen	-0,701493572
Lime solution in different concentrations and planting with basil	Non-spore forming bacteria	-0,734221392
	Bacilli/Lactobacilli	-0,795620966
	Actinomycetes	-0,727778137
	Micromycetes	-0,943242218
	Bacteria, assimilating mineral nitrogen	-0,690628129

With the non-pathogenic microflora are obtained stronger correlation connections between the quantity of the microorganisms and the different methods for treatment. The strong dependency with Micromycetes makes an impression. With them the coefficient of correlations as per absolute value is bigger than 0,9. Another dependency regarding the coefficients of correlation is noticed. Likewise with the pathogenic microorganisms and here the correlation connections are similar regardless whether it is studied only with presence of lime solution or it is planted additionally with lavender or basil. After establishing a strong correlation between the quality and quantitative treatment of the sludge and the quantity microflora in them, can be passed to creating a mathematical model for the experimental data.

Regression analysis

The purpose of the linear regression analysis is obtaining a mathematical model for the quantity of pathogenic microflora in sludge processed only by lime solution and such processed by lime solution and planted with lavender or basil. Here as factors except for lime solution concentration are included temperature, humidity and pH of the sludge. The regression analysis is carried out with the help of MS Excel. The results are classified in the following groups:

- Coefficient of multiple correlation; coefficient of multiple determination, which shows the percent from the total dispersion of the dependent variable, which is "explained"by the linear regression and etc.;
- Stages of freedom; regression sum of the squares; residual sum of the squares; Significance F is P- the value for the checkup of the statistical significance of the regression and etc.;
- Table with values of the coefficients in the regression equation, as well as statistic values for testing of these coefficients: a standard mistake of the coefficient, value of the t-statistics for testing of the statistical significance of the respective regression coefficient, the p-value, out of which we draw a conclusion whether the respective regression coefficient is statistically significant, i.e. whether it is different from 0, the lower and upper limit at 95% confidence interval the free term and the regression coefficients.

A main checkup is carried out as per P-value –the p-value. Must P<0,05.

Dependent variable Y: quantity of the respective type of pathogenic microflora

Independent variables: X1 - temperature, X2 - humidity, X3 - pH and X4 – concentration of the lime solution

Table 9 Data for temperature, humidity and pH of the sludge during the experiments

Variants	Temperature (°C)					Humidity (%)					pH (in water)				
	Before setting the experiment	1-st day, 6 hours after	5-th day	20-th day	40-th day	Before setting the experiment	1-st day, 6 hours after	5-th day	20-th day	40-th day	Before setting the experiment	1-st day, 6 hours after	5-th day	20-th day	40-th day
Sludge without lime	20,2	20,4	22,0	24,8	26,4	90,0	89,7	84,8	55,7	38,4	6,88	6,88	6,90	6,89	6,90

For all types of microflora, the procedure as per creation of a mathematical model begins with carrying out a regression analysis with participation of all independent variables. In some of the cases for a part of the independent variables are obtained P-values for some of the coefficients, higher than the limit value (P<0,05). In the presented equations these variables are eliminated from the mathematical model. In figure 2 can be seen presentation of the graphic results for each of the microflora types.

In the present publication are not presented all obtained results from the regression analysis, and only the graphic presentation of the obtained regression model in comparison with the experimental data and the respective remainders. With inspection of the results about the quantity *Escherichia coli and coliforms* the data seems well regarding P value, but big remainders are obtained. This may mean that the mathematical model is incomplete and for a better description of the dependent variable it would be better to be included and other factors.

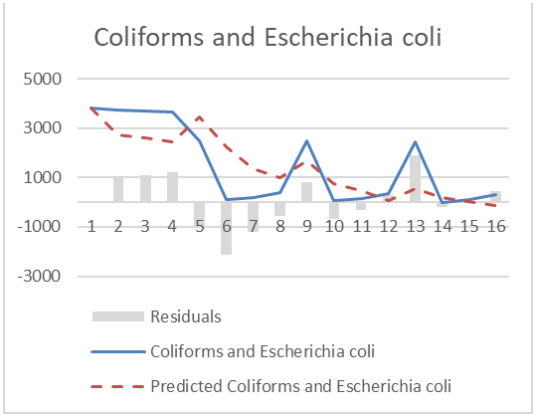
The biggest remainders are noticed with drawing up of a mathematical model for *Escherichia coli and coliforms* in sludge, treated only with lime solution. A similar tendency, however, is observed to a certain degree and when lavender or basil is planted. The graphic data gives even better idea. The obtained mathematical models for *Escherichia coli and coliforms* (the data for Predicted in figure a,b,c) to great degree repeat the experimentally obtained results, but the presence of big remainders speaks of an insufficiently reliable and complete model.

On the grounds of the obtained values for P it turns out that a part of the variables are not significant from the statistic point of view and it shall not participate in creation of the model. With description of the quantity *Enterococcus* in sludge treated with lime this is the concentration of the lime solution. With planting lavender or basil this result is confirmed. The quantity of *Clostridium perfringens* also turns out not to show a statistic connection with the percent content of the lime solution. Unfortunately, here, as well as with the previous two types of pathogenic microflora, are observed significant remainders with creation of the mathematical model. In this respect the results with the non-pathogenic microflora are quite different. With all five types of non-pathogenic microflora the obtained remainders are with small values, and the graphic results show a very good coincidence of the mathematical model and the experimental results. This tendency is very well seen in the graphic presentation of the models in figure 2.

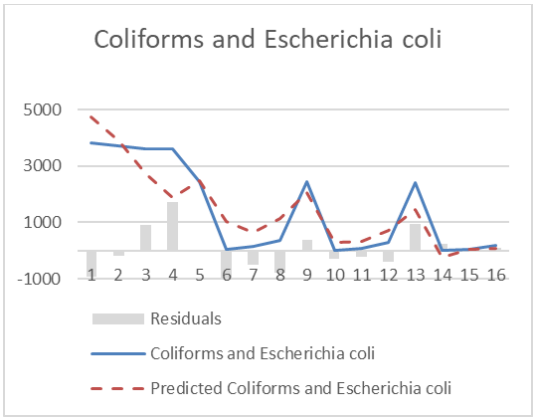
With creation of a mathematical model of non-spore-forming bacteria, the independent variable, which drops out from the mathematical model on the grounds of the obtained value for P is X3 – pH. From statistic point of view, it turns out that the change of the humidity does not render impact on the quantity of this type of microflora. The obtained graphic results give reasons to be affirmed that on the grounds of the rest variables is created a good mathematical model.

Even better coincidence between the mathematical model and the experimental data is observed with bacilli/lactobacilli, actinomycetes, micromycetes. With them the remainders have minimum values, and the graphics of the experimental data and the obtained from the regression analysis (Predicted) have almost complete coincidence. It is interesting to be noted that while with creation of mathematical model of the quantity of actinomycetes in the sludge participate almost the whole set of variables (with planting lavender and basil lacks one), with the rest two types of non-pathogenic microflora the model consists of decreased number of variables. The common with bacilli/Lactobacilli and micromycetes is that with the creation of their regression equations lacks the variable X1 – temperature. The value of P for it is obtained outside the recommended limit and it drops out in the final form of the regression equation.

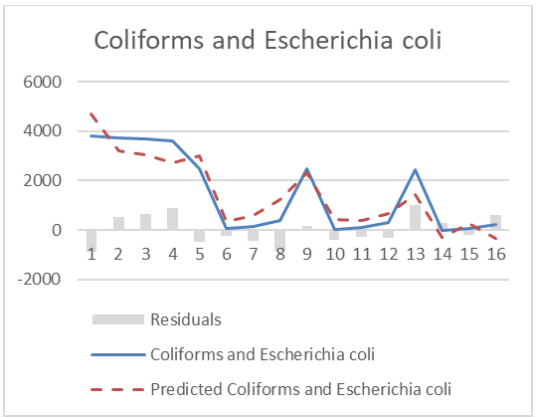
When describing the last type of non-pathogenic microflora - bacteria that absorb mineral nitrogen statistically significant turn out to be the variable temperature, humidity, and percent content of the lime solution. They participate in the regression equation with all the three considered types of sludge – treated only with lime solution, treated with lime solution, and planted with lavender or basil.



a) *Escherichia coli* and coliforms in Sludge + CaO $Y = 63,4*X_2 - 271,3*X_3$

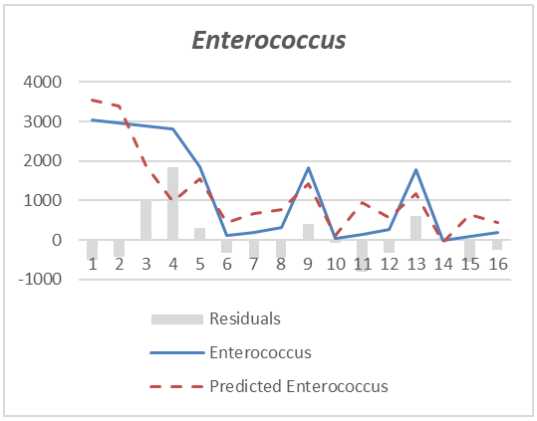


b) *Escherichia coli* and coliforms in Sludge + CaO + Lavandula $Y = 18889,7 - 513*X_1 - 520,9*X_3$

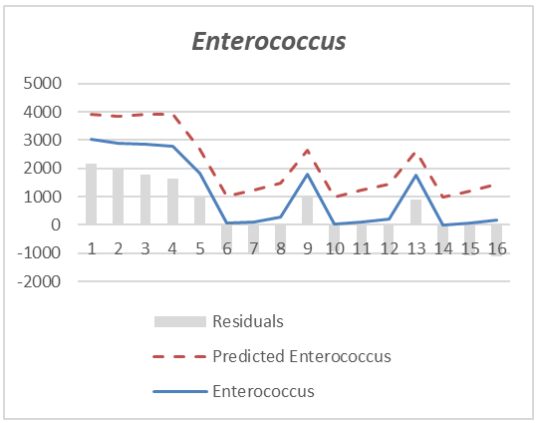


c) *Escherichia coli* and coliforms in Sludge + CaO + Ocimum

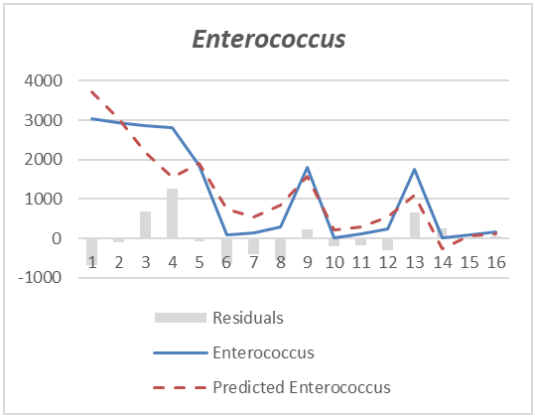
$Y = 39366,8 - 1180*X_1 - 87,7*X_2 - 343,9*X_3$



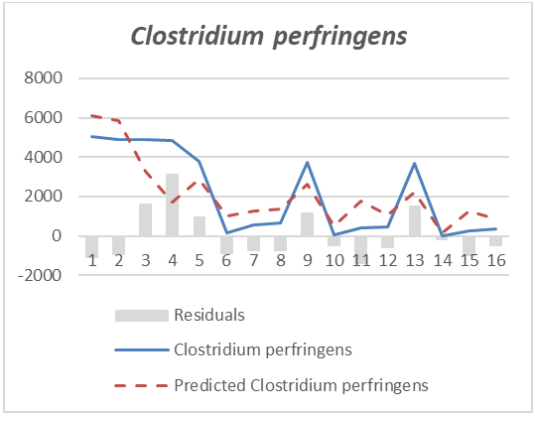
d) *Enterococcus* in Sludge + CaO $Y = 83,8*X_1 + 59,8X_2 - 512,4\ X_3$



e) *Enterococcus* in Sludge + CaO + Lavandula $Y = 48,3*X_1 - 16,9*X_3$

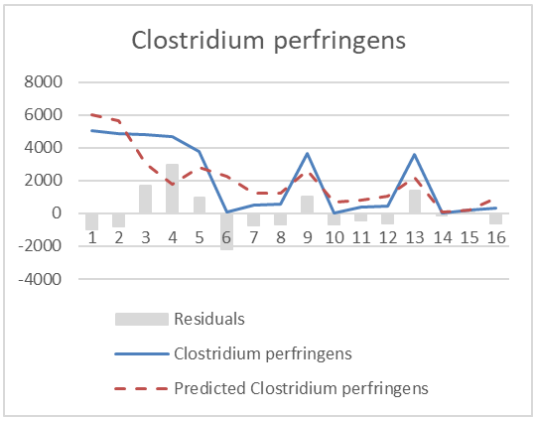


f) *Enterococcus* in Sludge + CaO + Ocimum $Y= 14761,7 - 392,9*X_1 -421,9*X_2$



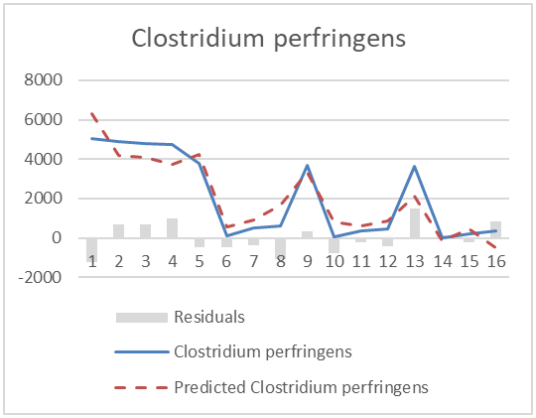
g) *Clostridium perfringens* in Sludge + CaO

$Y=136,5*X_1+101,4*X_2-836,2*X_3$



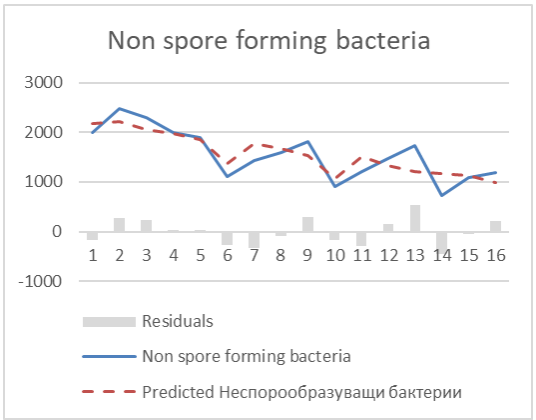
h) *Clostridium perfringens* in Sludge + CaO + Lavandula

$Y=5329,4-1632,8*X_1-120,1*X_2-390,9*X_3$



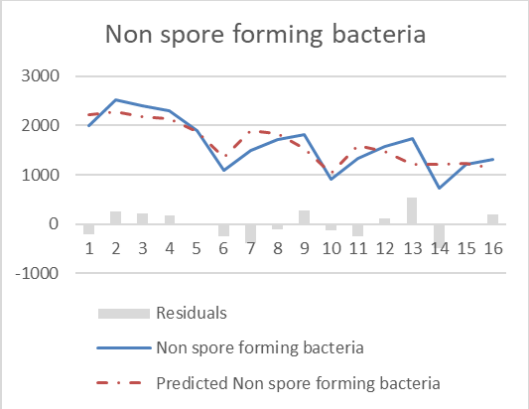
i) *Clostridium perfringens* in Sludge + CaO + Ocimum

$Y=54274,9-1942*X_1-125,2*X_2-400,5*X_3$



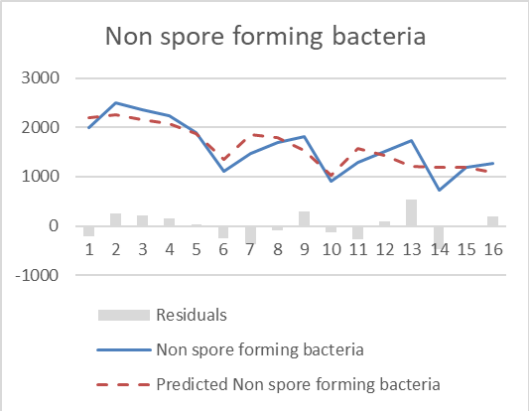
j) Non-spore-forming bacteria in Sludge + CaO

$Y=59*X_1+10,8*X_2-35,5*X_4$



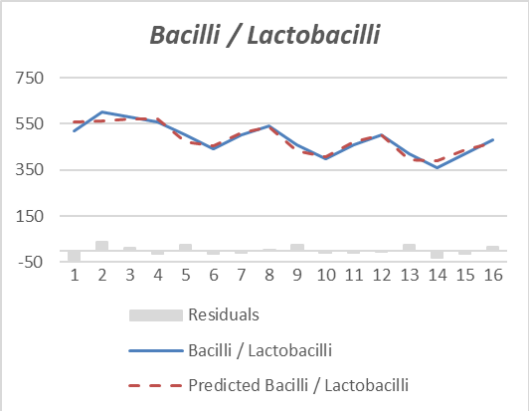
k) Non-spore-forming bacteria in Sludge + CaO + Lavandula

$Y=67*X_1+9,4*X_2-37,5*X_4$

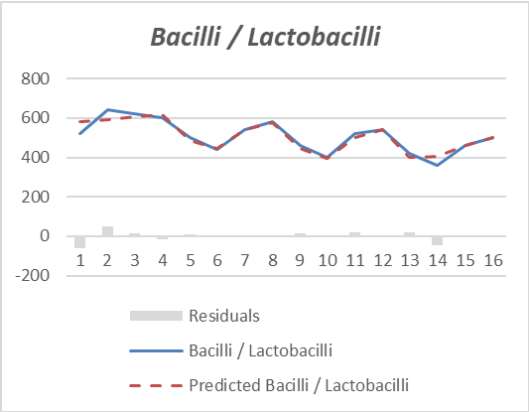


l) Non-spore-forming bacteria in Sludge + CaO + Ocimum

$Y=64,8+9,8*X_2-37,1*X_4$

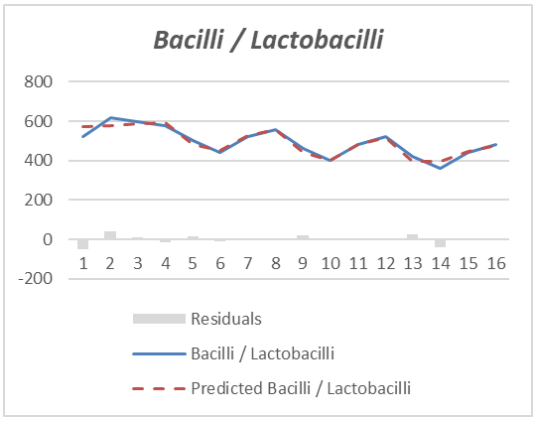


m) Bacilli / Lactobacilli in Sludge + CaO $Y=690-17,8*X_3-2,9*X_4$



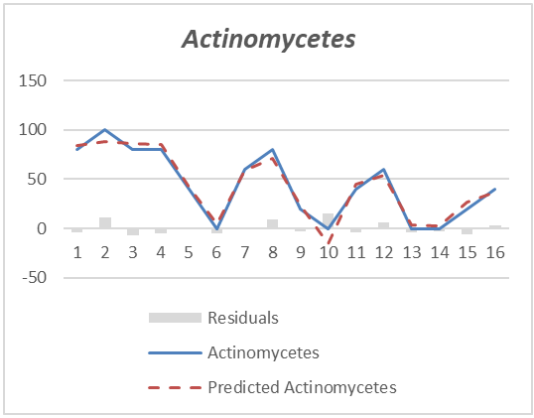
n) Bacilli / Lactobacilli in Sludge + CaO + Lavandula

$Y=778,6-25,6*X_2-2,5*X_4$



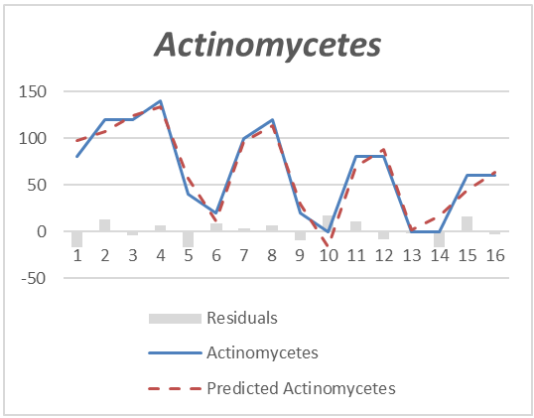
o) Bacilli / Lactobacilli in Sludge + CaO + Ocimum

$Y=728,4-20,9*X_3-2.9*X_4$



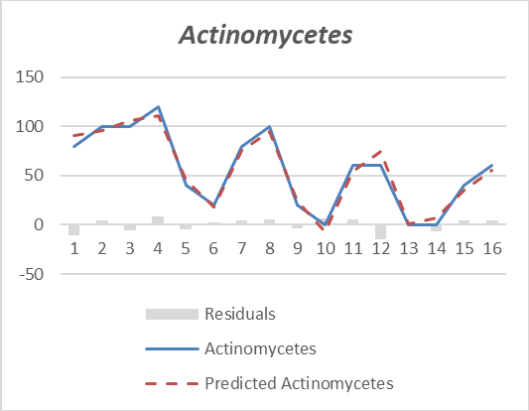
p) Actinomycetes in Sludge + CaO

$Y=4,3*X_1+0,5*X_2-6,9*X_3-1,8*X_4$



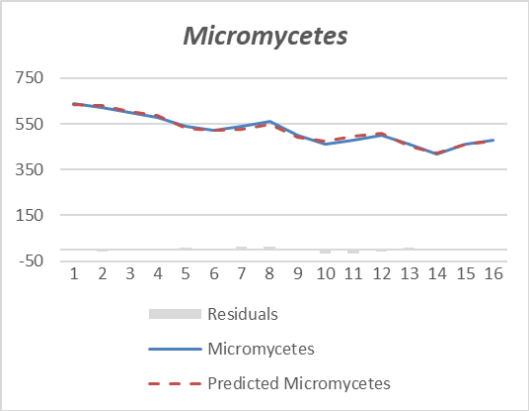
q) Actinomycetes in Sludge + CaO + Lavandula

$Y=6,1*X_1-3,9*X_3-2,9*X_4$



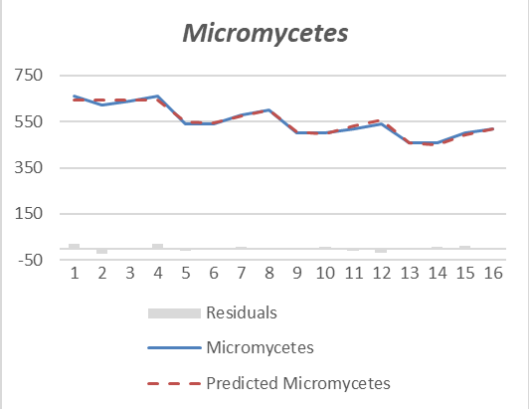
r) Actinomycetes in Sludge + CaO + Ocimum

$Y=65,6+3,5*X_1-6,7X_3-2,1*X_4$



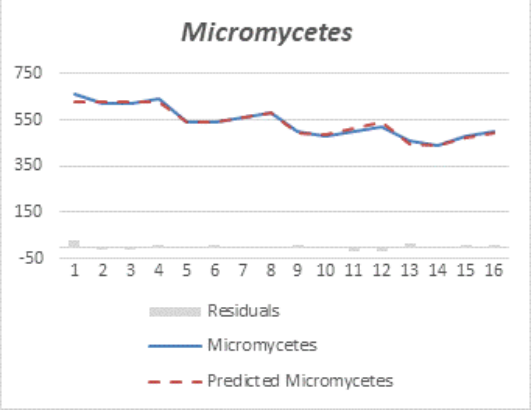
s) Micromycetes in Sludge + CaO

$Y=672,9+0,9*X_2.17,9*X_3-3,2*X_4$



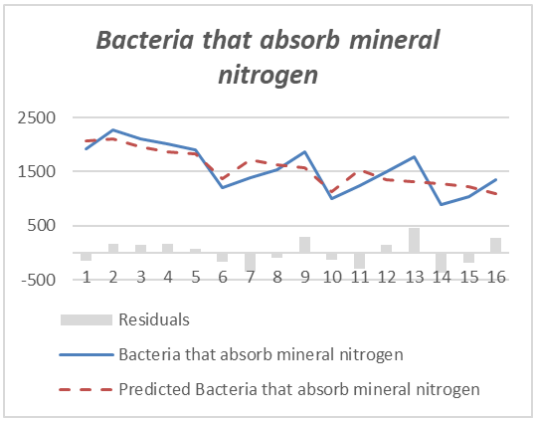
t) Micromycetes in Sludge + CaO + Lavandula

$Y=733,1-13,2*X_3-3,9*X_4$



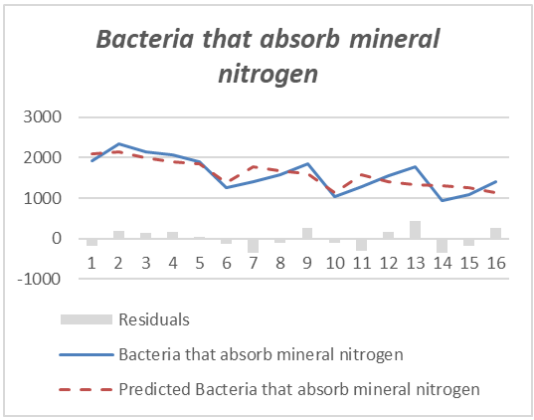
u) Micromycetes in Sludge + CaO + Ocimum

$Y=704,1-10,7*X_3-4,4*X_4$



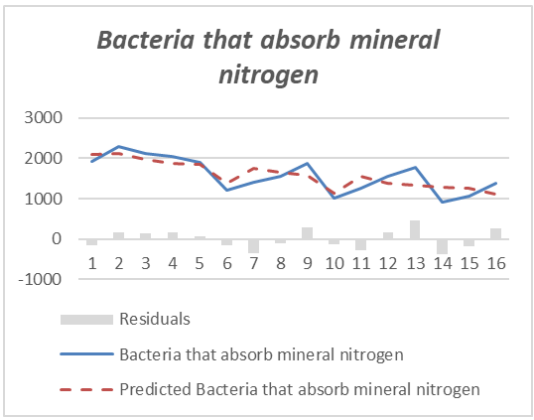
v) Bacteria that absorb mineral nitrogen in Sludge + CaO

$Y=55*X_1+10,7*X_2-28,4*X_4$



w) Bacteria that absorb mineral nitrogen in Sludge + CaO + Lavandula

$Y=56,9*X_1+10,5X_2-28,7*X_4$



x) Bacteria that absorb mineral nitrogen in Sludge + CaO + Ocimum

$Y=55,5*X_1+10,5*X_2-28,4*X_4$

Figure 2 Graphic results for the mathematical models of the different types of microflora.

DISCUSSION

Microorganisms are sensitive indicators of the changing conditions of the environment (**Bending et al., 2000, Bruggen-Van et al., 2000, Poudel et al., 2002, Panikov, 1999, Schinner et al.,1996, Hyman et al., 1990, Morfenina, 1996, Torsvoc et al., 1996, Zenova et al., 1998, Nustorova et al., 2000, Malcheva, 2020, Caravaca et al., 2002, Saggar et al., 2020**). Their modelling is a challenge, requiring a long-term and multi-factor study in dynamics. While it is being worked on the creation of methods for the disinfection of the sludge significantly, it is not paid attention for following the useful microflora during the treatment. The juxtaposition of pathogenic and non-pathogenic microflora

development in a medium influenced by identical factors may contribute for modelling an effective system „disinfection-utilization"of sludge with purpose their safe and quality usage as fertilizer in agriculture. In the studied sludge the highest is the quantity of the non-spore forming bacteria from the non-pathogenic microflora and of the clostridia from the pathogenic microorganisms. The lime treatment has a favorable effect for the disinfection of the sludge, but at the same time leads to decrease of the useful microflora. The microbiological analysis of the results was presented in a previous scientific material (**Malcheva et al., 2021**).

The results, obtained from the statistic processing of the experimental data give opportunity to be drawn some conclusions regarding the influence of the separate factors (independent variables) on the quantity of pathogenic and non-pathogenic microflora. The difference in the obtained remainders with both groups' microflora is clearly noticed. There are comparatively big shifts in the graphic results between the regression equations and the experimental data with all types of pathogenic microflora. This may speak of insignificant available data for creation of a mathematical model. On the one hand, it is possible there to be quite few observations, but the reason may be also the impact of additional factors, which in the case are not reported.

In all regression equations for the pathogenic microflora lacks X4 – concentration of the lime solution. These results show that the concentration of the solution does not influence significantly on the quantity pathogenic microflora with a regression analysis. On the other hand, the results from the preliminary dispersion analysis show a strong correlation dependency between the concentration of the solution and the quantity of microorganisms. The reason for obtaining this discrepancy may be the necessity of adding additional factors with carrying out a regression analysis for pathogenic microflora. The adding of lime to sludge from a purification plant increases the values of pH, which leads to destruction of the pathogenic microorganisms. Depending on the content and quantity of the pathogenic microflora the effective concentration of lime may be different and also to depend on factor time, i.e. on necessity of repeated lime treatment (**Malcheva et al., 2021, Malcheva et al., 2022**). Different authors present methods for treatment of pathogenic microorganisms in sludge with different concentrations. According to Wong and Fang (**Wong et al., 2000**), the adding of 0,63% lime before composting the sludge slightly improves the microbial activity of temperature increase and release of CO2 and does not inhibit significantly the bacterial population after 100 days since composting. On the one hand, this concentration inhibits in small degree the development of useful groups of microorganisms in the sludge, but according to **Marinova et al**., 2016 most perspective for a short period of time (around 1 month) is the disinfection of the sludge by adding 20% fine and 30% rougher quicklime fraction. According to them the treatment with quicklime is especially appropriate for this purpose, since lime is not dangerous for the environment and in the same time it is effective. **Popova et al., 2014** have established that the application of 28% CaO is enough per one month for destruction of sanitary-indicative microorganisms. These authors indicate that the application of quicklime in different concentrations and for different time depends on the types and quantity of the identified microorganisms – higher concentrations of CaO (33-38%) should be applied at presence of big quantities dangerous microorganisms in the sludge. According to **Malcheva et al., 2021, Malcheva et al., 2022** best results for disinfection of the sludge shows the adding of 20% and 30% quicklime for the period 5-th-10-th day from the experiment. After 20-th-25-th day, however, the values of pH fall to neutral and alkalescent medium, which leads to repeated development of pathogenic microorganisms (especially spore forming like *Clostridium perfringens*) and respectively a repeated lime treatment is necessary.

Reporting factor time is necessary, including for a period after repeated lime treatment until a complete and long-term destruction of the pathogenic microorganisms, which shall increase the period of observation and respectively the number of the experimental data. The model for the pathogenic microorganisms can be supplemented with reporting the impact of other factors (independently or in a combination), decreasing the quantity of the pathogens: adding of ashes, microbial fertilizer and vegetation with antimicrobial activity, ammonia, formaldehyde, extreme low or high temperatures (**Malcheva et al., 2021, Malcheva et al., 2022, Popova et al., 2014, Mendez et al., 2004**).

When creating the regression equations for the quantity non-pathogenic microflora in the sludge, a very good coincidence between the experimental data and the obtained models is seen. With them the remainders are much smaller. The concentration of the lime solution turns out to be a significant factor. This independent variable participates with formation of the regression equations for all variants with non-pathogenic microflora – treatment with lime, treatment with lime and planting with lavender or basil. Regarding the non-pathogenic microflora the results from the regression analysis coincide with these from the preliminary correlation analysis. This gives grounds to be reckoned that the obtained mathematical models can be used with prognostication the content of the considered types of microflora in studied sludge. The increase of the quicklime concentration destroys to a higher degree the pathogenic, as well as the non-pathogenic microorganisms, which imposes the searching of balance when transforming the sludge into a safe, but effective fertilizer.

With considering the regression equations of the non-pathogenic microflora for two of the groups of microorganisms - Bacilli / Lactobacilli and Bacteria that absorb mineral nitrogen, in the equations participate less in number variables. With them the temperature does not go into the mathematical model, in contrast with the pathogens, where it is a significant factor. The change in the temperature is a factor assisting the disinfection of the sludge. The microorganisms do not have mechanisms for regulation of their own temperature and on principle are strongly impacted by the temperature changes. The bacilli as well as *Clostridium perfringens*, as a spore forming microorganisms survive to a higher degree with changing conditions of the environment. In the present study the sludge is not put at extreme minus or plus temperatures, as well as a wide temperature interval, but even increase of the temperature with around 9 °C has a positive effect for disinfection of the sludge from pathogenic microorganisms, as in the meantime this increase does not decrease the biogenicity of the non-pathogenic microflora. The optimum temperature for development of the studied non-pathogenic microorganisms (27 °C – 28 °C) corresponds to the sludge temperature (20,4 °C – 29,4 °C), while the analyzed pathogens are cultivated at higher temperature (around 37 °C), which is also a precondition for a stronger dependency between the temperature of the sludge and the pathogens.

The usage of plants with antimicrobial activity may contribute for disinfection of the sludge from pathogenic microorganisms. In the present experiment the application of lavender and basil leads to decrease in the quantity of the pathogenic microflora and preservation of the useful microorganism's development, as the effect is almost identical with independent usage of one of both plants. It is of interest the model to be complemented by a study on the combined effect impact of both plants, as well as planting other plants. The plants synthesize flavonoids as a response of a microbial infection and it is established that these compounds are a mighty antimicrobial agent against a wide spectrum of pathogenic microorganisms (**Górniak, et al., 2019**). In this relation the study on antimicrobial properties of plants at variants with sludge from purification plants, treated for disinfection and deodorizing deserves attention.

Pictures of some isolated microorganisms in the sludge are presented in Figure 3.

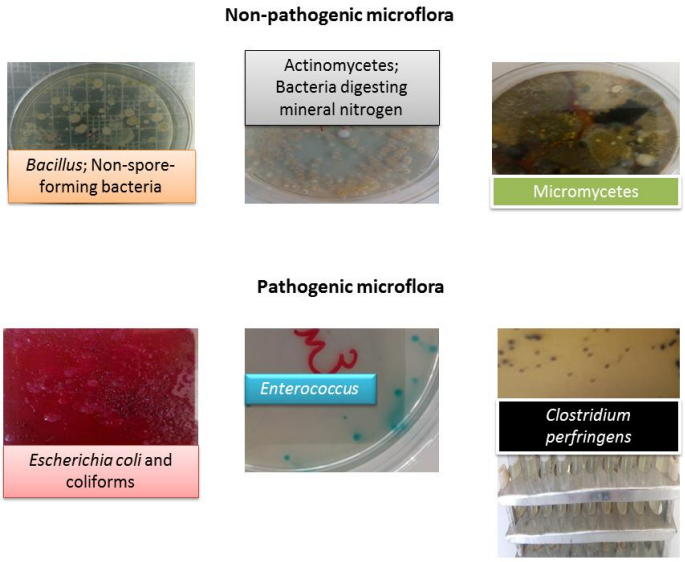


Figure 3 Isolated microorganisms

CONCLUSIONS

The data from the experiments are processed via statistic methods. Firstly, is carried out a correlation analysis for finding out dependencies between the concentration of the lime solution and the quantity of microorganisms. High coefficients of correlation are established. Then a regression analysis for creation of a mathematical model for the quantity of microflora in sludge treated in a different way is carried out – only with lime solution, with lime solution and planting of lavender or basil. The following conclusions may be drawn on the grounds of the obtained results:

- The different methods for treatment render greater impact on the presence of non-pathogenic microflora. This is proven by the obtained bigger coefficients of correlation at carrying out a correlation analysis.
- The planting of the sludge with lavender or basil does not change significantly the statistic coefficients and dependencies. Both plants can be used equivalently independently or in a combination with disinfection and deodorizing of sludge.
- Additional experiments are necessary for creation of an appropriate mathematical model of the quantity pathogenic microflora in sludge. The usage of other statistic procedures also would give better results. The search of a better mathematical description of this type of microflora is a task for subsequent developments of the team.
- The obtained regression equations for the non-pathogenic microflora would be used for description and quantitative determination of the studied types of microorganisms in the sludge.
- Taking into account the high and divergent sensitivity of the microorganisms in an extreme medium, as the sludge from a purification plant is, is necessary the analyzing in dynamics of pathogenic and non-pathogenic microflora interrelatedly with disinfection of the sludge with purpose safe and effective usage as fertilizer in agriculture.

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Conflicts of Interest: The authors declare no conflict of interest.

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