SOFIA UNIVERSITY
St. KLIMENT OHRIDSKI

RELATIONSHIPS BETWEEN BACTERIAL SEGMENT AND FAUNA COMPLEXES IN ACTIVATED SLUDGE AS AN INDICATIVE APPARATUS FOR TREATMENT OF LEACHATE FROM LANDFILL

КОПХ
АСОЦИАЦИЯ ПО ОБЩА И ПРИЛОЖНА ХИДРОБИОЛОГИЯ

INTERNATIONAL SCIENTIFIC CONFERENCE "KLIMENT'S DAYS" 2020

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Abstract Landfills can be sources of various types of water pollutants. As the amount of waste in their mixed collection increases, so do the toxic compounds in it. One of the main problems for biotechnologists in wastewater treatment plants remains the control of the effective biodegradation of incoming pollutants from the leachate. The aim of this study is to develop an indicative apparatus to control the treatment of infiltrate from landfills through the relationship of bacterial segment and fauna complexes in activated sludge. To achieve this goal, a model adaptation process of activated sludge to leachate from a landfill will be carried out. The biodegradation potential of the bacterial segment is strongly inhibited due to the contained "bouquet" of xenobiotics (toxic organic and inorganic contaminants) in the leachate. Fauna complexes, which have an extremely important supporting role in activated sludge, are severely suppressed and in most cases die. This may be related to their function as a "protector" and xenobiotic distributor for bacteria. This is evidenced by studies in which the biotic index of micro- and metafauna decreases at the end of the experiment. From a maximum value of 10 in the middle of the process, to a value of 1 at the end of the process. In addition, there is a sharp decrease in the diversity of macroorganisms. All this is combined with relatively high amounts of microbiological indicators for aerobic heterotrophs, bacteria of the genus *Pseudomonas* and the genus *Acinetobacter*, in the middle and end of the experiment. The main hypothesis of the study is that the control of wastewater treatment processes can be achieved by indicating and regulating the relationship between bacteria and micro- and metafauna, on a quantitative and qualitative level. Studying these micro relationships between pro- and eukaryotic organisms would help to overcome the possible inhibition of wastewater treatment processes due to the toxic substances contained in the leachate from landfills.

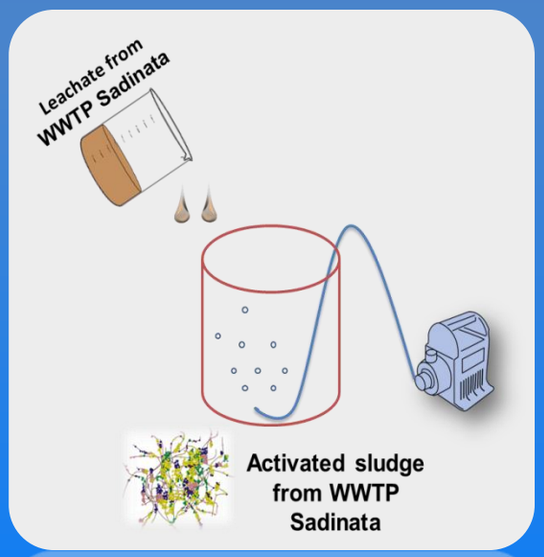
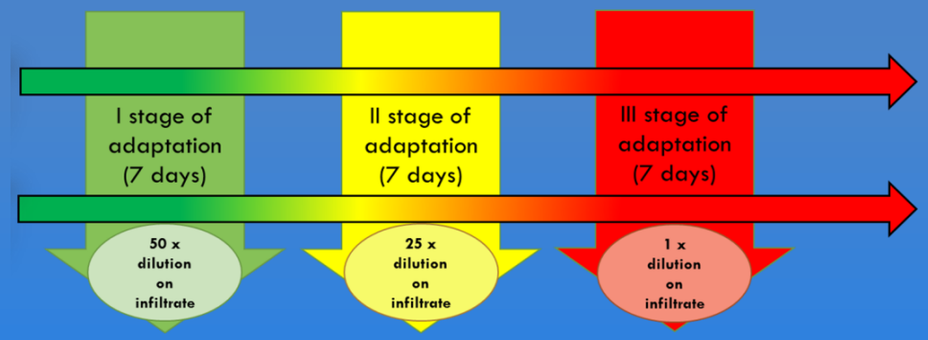


Fig. 1 Design of experiment



During the experiment it was constructed a model of a bioreactor - type "aerobic bio-basin". The treatment process was started with an activated sludge from WWTP "Sadinata" and an addition of a leachate. The water treatment process was started with a diluted leachate as its concentration was increased step by step during 3 weeks. The aim is to be achieved an adaptation of the activated sludges with a step by step increase of the concentration of the pollutants (Fig. 1).

Table 1. Investigated parameters and methods for analysis of activated sludge and wastewater

Parameter	Method	Description of method
Sludge biotic index (SBI)	Madoni, 1994	Microscopic examination of qualitative and quantitative characteristics of microfauna in activated sludge.
Biodiversity of micro- and metafauna	Foissner and Berger, 1996	Microscopic examination of number of taxa and quantity of different groups of organisms from micro- and metafauna in activated sludge.
Aerobic heterotrophic bacteria (AeH)	Topalova, 2009	Used classical cultivation method for determining the number of aerobic heterotrophs by surface plating with 0,1 ml of the sample by appropriate dilution in a Petri dish with a solid agar.
genus Pseudomonas and genus Acinetobacter	Topalova, 2009	Used classical cultivation method for determining the number of bacteria by surface plating with 0,1 ml of the sample by appropriate dilution in a Petri dish with a solid agar.
Chemical oxygen demand (COD)	ISO 6060:1989	The color transition is from blue-green to reddish-brown. An equivalent point is the first sharp change in color from blue-green to reddish-brown.

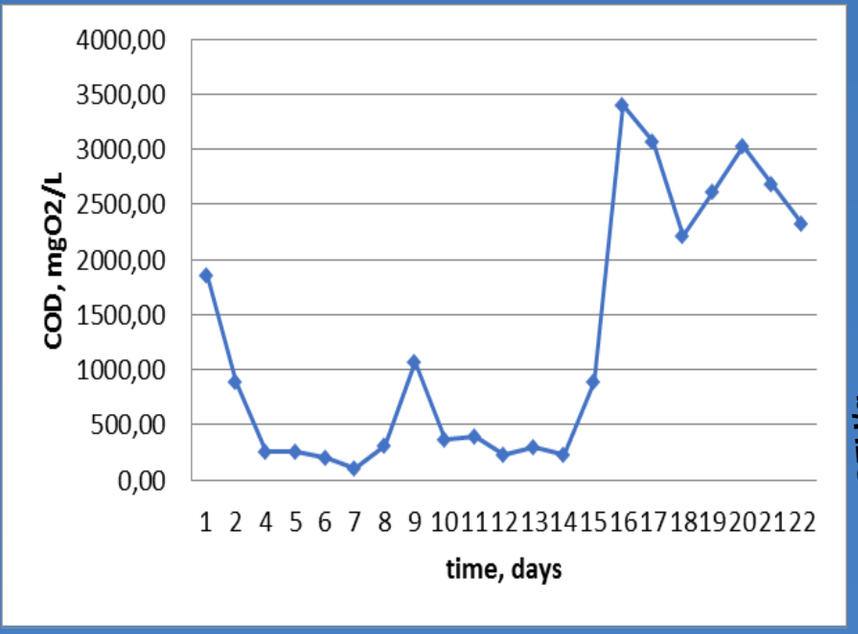
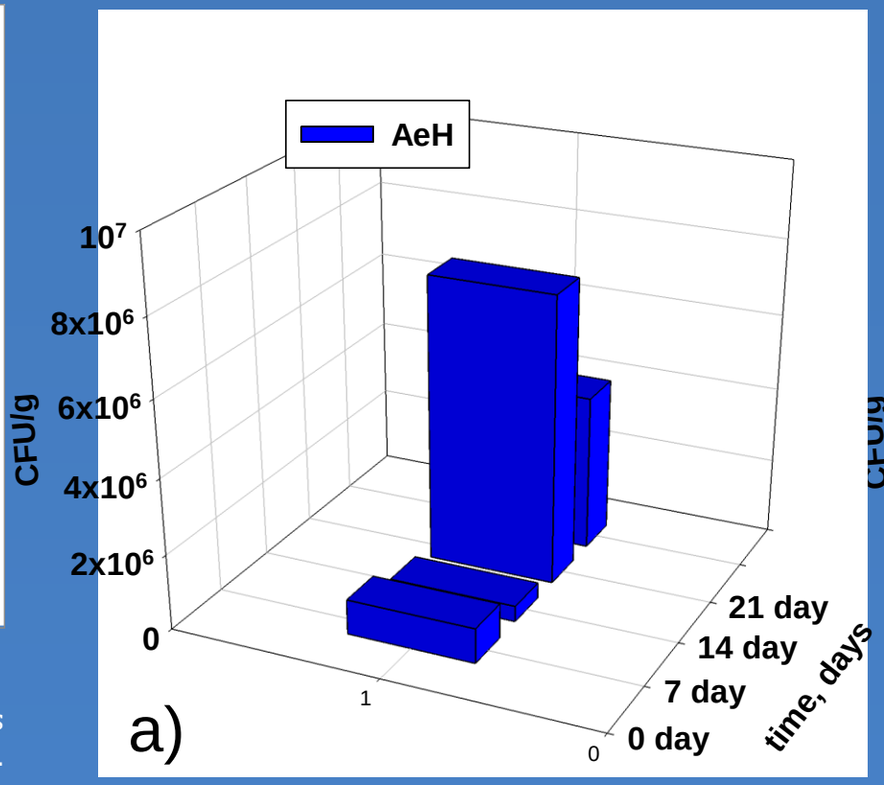


Fig. 2 Dynamics of COD during the process.

In the beginning of the process the COD of the effluent was decreased from 1858,28 mgO_2/L to 258,23 mgO_2/L (Fig. 2). The activated sludge adapted towards biodegradation of the landfill leachate which was diluted 50 times. When the concentrations of the pollutants was raised (25 times diluted leachate) the COD of the effluent increased up to 1064.38 mgO_2/L due to the higher toxicity and suppression of the bacterial activity. Up to the fifteenth day the COD was decreased again to 890,55 mgO_2/L because the community low adapted to biodegradation of the applied leachate. When undiluted landfill leachate was used the COD of the effluent reached 3035,65 mgO_2/L. The reason for the registered effects was the strong intoxication leading to community disintegration.



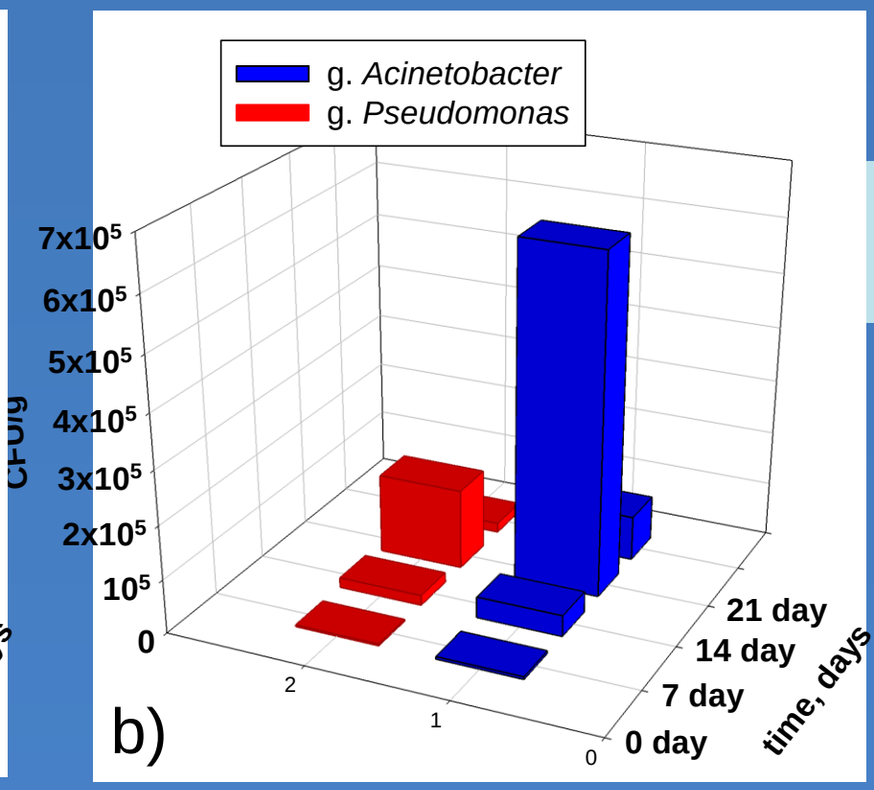


Fig. 3 Quantity of aerobic heterotrophic bacteria (a) and quantity of bacteria of the genus *Pseudomonas* and bacteria of the genus *Acinetobacter* (b)

Aerobic heterotrophic bacteria have increased in number by the time of addition infiltrate without dilution (14 day) (Fig. 3a). The highest amount of heterotrophs is reported on 14 day. Then, the bacteria are probably most adapted to the biodegradation of the infiltrate. In the bacteria of the genus *Pseudomonas* and the genus *Acinetobacter*, also recorded an increase of their quantity by the time of addition to the system of the infiltrate without dilution (Fig. 3b). At the end of the study process, the quantities of both groups dropped very sharply. At the end of the process, the bacteria of the genus Acinetobacter are several times larger than the pseudomonads.

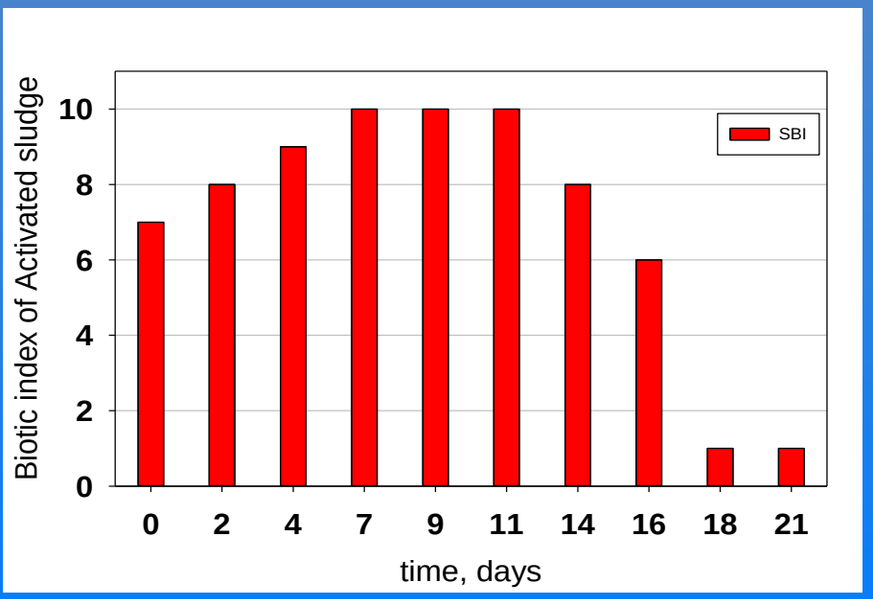
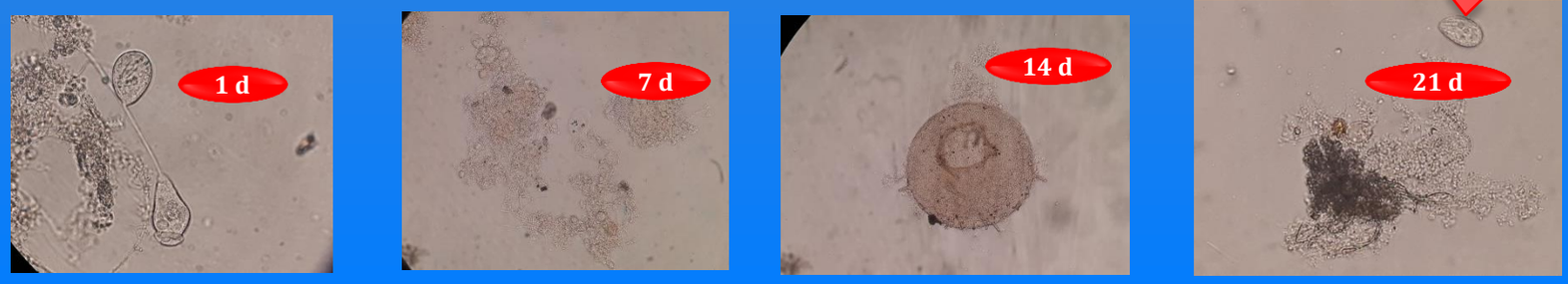


Fig. 4 Biotic index of activated sludge (Madoni, 1994)

By the data of the biotic index it is established a tendency for its decrease at the end of the process. In the model with the activated sludge from the waste treatment plant it is registered a decrease in this index in the second and third stage of adaption. (14 day – 8 and 21 day – 1). During the last stage of adaption, after the addition of a leachate without any dilution, the communities of the micro- and metafauna react with a sudden change of the dominant groups. The dominant groups are small flagellates and **free-swimming ciliates**. Their large number leads to a decrease in the biotic index of activated sludge. This is mostly a type of an adaptive reaction of the key groups of the micro- and metafauna in the presence of xenobiotics in larger quantities.

Table 2. Microscopic images of the activated sludge (400x)



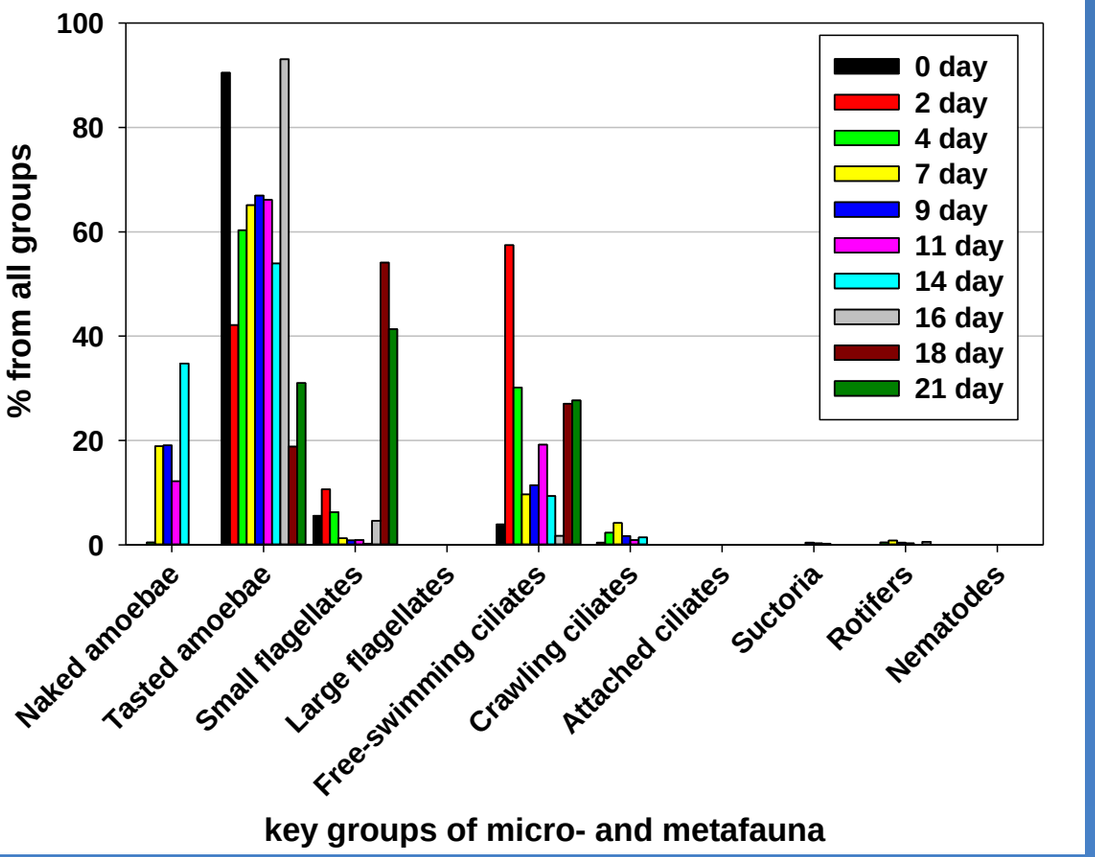


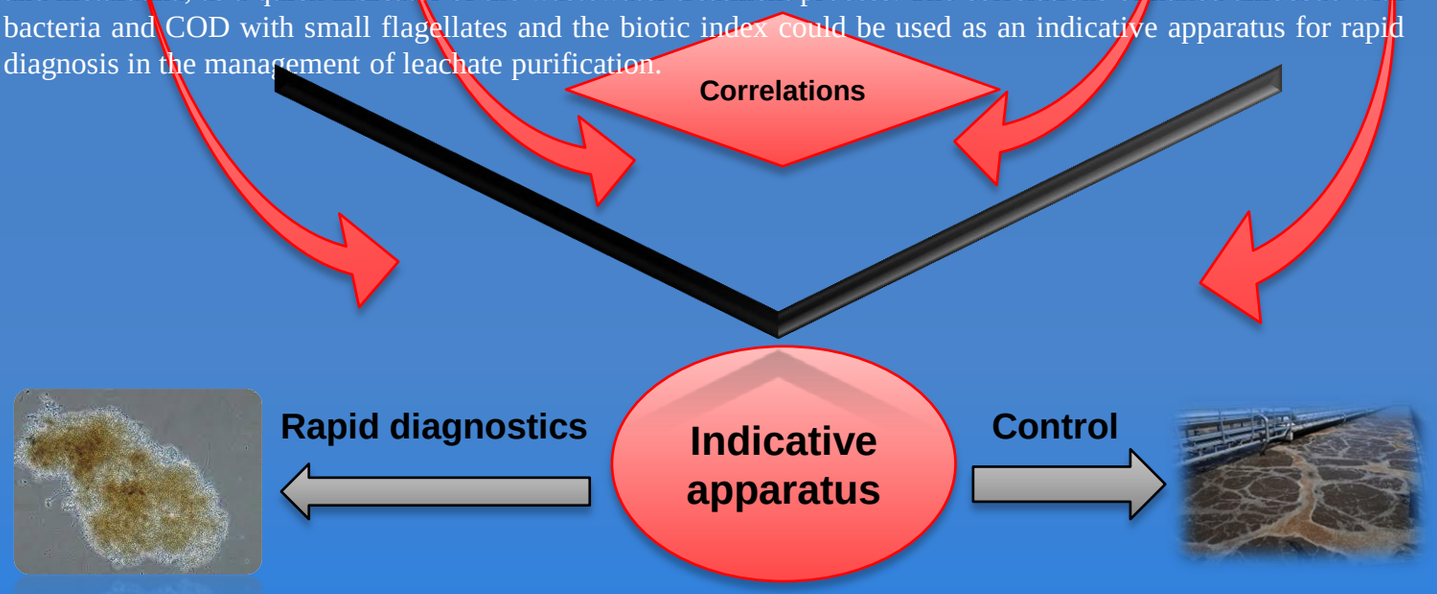
Fig.5 Percentage distribution of key groups of micro- and metafauna

The percentage distribution of micro- and metafauna organisms shows that the tasted amoebae and free-swimming cilliates dominate in the initial stage. In the second stage, in addition to the mentioned group, a high percentage is also observed in the small flagellates, which directly leads to the deterioration of the quality of the activated sludge. After the second stage, the dominant key groups are tasted and naked amoebae. At the end of the study process, small flagellates again had the highest percentage, but together with free-swimming ciliates. These two dominant groups are indicators of deterioration of the quality of the activated sludge, which is a fact in the third stage after the addition of infiltrate without dilution. Very important for the trophic and macro structure of the activated sludge is the small quantity of crawling ciliates and the complete absence of attached ciliates during the whole studied process. Their absence is an indicator of a deteriorating wastewater treatment process.

Table 3. Correlation between hydrochemical, microbiological parameters and quantity of key groups of micro- and metafauna in activated sludge.

	Naked amoebae	Tasted amoebae	Small fllagelates	Swimming Cilliates	Crawling cilliates	Rotifers	SBI	COD
Aerobic Heterotrophic Bacteria	R^2=0,59	P	P	P	P	P	P	P
g. *Acinetobacter*	R^2=0,92	P	P	P	P	P	P	P
g. *Pseudomonas*	R^2=0,96	P	P	P	P	P	P	P
COD	P	P	R^2=0,65	P	R^2=0,91	P	R^2=0,80	-

The linear correlation observed between naked amoebae and aerobic heterotrophs (R^2=0,59), bacterium (R^2=0,92) and bacterium (R^2=0,96) in the activated sludge confirms the indicator role of amoebae in the deterioration of the purification process. Although they do not play a dominant role in the studied process, amoebae have a pronounced diet with bacteria. In the presence of toxic substances, naked amoebae and small flagellates are the ones that have the strongest endurance. These indicator connections are important for wastewater of a toxic nature, such as leachate. There is a linear correlation between COD and small flagellates (R^2=0,65), creeping ciliates (R^2=0,91) and the biotic index (R^2=0,80) in the activated sludge. In conditions of highly toxic wastewater, small flagellates are a known indicator, as evidenced by the correlation with COD. The linear relationship between the biotic index and the COD confirms the importance of the key groups of micro- and metafauna, as a quick indicator of the wastewater treatment process. The correlations of naked amoebae with bacteria and COD with small flagellates and the biotic index could be used as an indicative apparatus for rapid diagnosis in the management of leachate purification.



Conclusions

In conclusion, it is established that the control and regulation of the wastewater treatment process can be assisted by an indicative apparatus obtained on the basis of correlations between bacteria and micro- and metafauna. The most significant correlations are those between naked amoebae and aerobic heterotrophs, *Pseudomonas* and *Acinetobacter*, COD and small flagellates and the biotic index. Further study of the relationships between pro- and eukaryotic organisms would help to overcome the possible inhibition of wastewater treatment processes due to the toxic substances contained in the leachate from landfills.

Acknowledgements

This work has been carried out in the framework of the National Science Program " Young scientists and postdoctoral students", approved by the Resolution of the Council of Ministers № 577/17.08.2018 and supported by the Ministry of Education and Science (MES) of Bulgaria (Agreement № Д01-322/18.12.2019).