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New Technologies of Microbiological Studies of Objects of the Environment

M.V. Nikolenko and Zh.B. Kostyrina
Industrial University of Tyumen, Street Volodarskogo 38, 625000 Tyumen, Russia

Abstract: In this study, the method of accelerated microbiological research using the express analyzer, registering changes in the impedance of the nutrient medium which occurs under the influence of growth processes and the vital activity of microorganisms in the test sample for studying the sanitary condition of soils in the city of Tyumen was proposed. The main advantage of this method is the automatic registration and processing of data which allows you to obtain objective results, reduce research time, reduce labor costs and significantly reduce the cost of analysis.

Key words: Impedance method, mesophilic aerobic and facultative anaerobic microorganisms, standard plate, sanitary-indicative bacteria, accelerated, influence

INTRODUCTION

Research problem: Soil is a natural biotope for many microorganisms. In the soil live and die various pathogenic bacteria, viruses, protozoa, helminth eggs. It is proved that the contaminated environment can directly or indirectly have toxic, allergenic, carcinogenic, mutagenic and other effects on the human body (Bolshakov, 2014; Kondakova, 2005; Gromov, 2010). A particularly dangerous role in regard of epidemics is played by fecal masses that inevitably enter the soil. Monitoring the cleanliness of the soil is important in the planning, construction and reconstruction of newly populated areas and populated areas, selection of sites for the construction of children's preschool institutions, sanatoriums, recreation areas, construction of water reservoirs, solving issues of water supply and sanitation. Current monitoring is necessarily conducted on epidemic grounds to find out possible ways of transmission of infectious agents. In addition to residential facilities, sanitary and epidemiological requirements are imposed on the territories of various plants and places of storage of industrial and domestic waste where the impact of contaminated soils on human health and living conditions is possible (Kamysheva, 2016).

Given the importance of the issue it makes sense to use universal and not time consuming methods of rapid analysis for the presence of a total number of microorganisms and pathogenic bacteria. In this study, we proposed a modern method of divided impedance which was successfully used for microbiological research and quality control and certification of food, raw materials, beverages, cosmetics, sterility control of various materials and solutions. The main advantage of this method is the automatic registration and processing of results which allows to obtain objective data and to shorten the time of analysis.

Aim of research: Apply new technological methods for microbiological research of the sanitary-bacteriological state of soil in the city of Tyumen.

MATERIALS AND METHODS

As the objects of the study, the following territories of the city of Tyumen were chosen: the Regional Clinical Hospital No. 1-OKB 1 (4 km Chervishevsky tract, 7) Sample No. 1, the park "Gilevskaya Roscha" (Gilevskaya Roshcha Street, 4) Sample No. 2, the Regional Clinical Hospital No. 2 OKB 2 (Melnikayte Street, 75) Sample No. 3, the Regional Blood Transfusion Station (Energetikov Street, 35) Sample No. 4, children's playground (Republic Street, 133) Sample No. 5, McDonald's Fast Food Supermarket (Lenin Street, 54) Sample No. 6, the Square of the 400th Anniversary of Tyumen (Republic Street) Sample No. 7, School No. 29 (Murmanskaya Street, 31) Sample No. 8, Alexander Moiseenko Garden Square (Odesskaya Street) Sample No. 9. All objects are classified according to sanitary and bacteriological indicators (Sanitary Rules and Regulations, SanPiN 2.1.7.1287-03) to areas of increased risk (Gromov, 2010). Sampling of soil was carried out according to the standard procedure (All Union State Standard GOST R 530912008 (ISO 10381-3: 2001), part 3 "Sampling") (Kondakova, 2005).

Samples of the soil were examined in a BacTrac 4300 analyzer by SY-LAB Gerate GmbH (Austria)

Corresponding Author: M.V. Nikolenko, University of Industrial Tyumen, Street Volodarskogo, 38, 625000 Tyumen, Russia

according to the methodological recommendations 4.2.2578-10 "Sanitary-bacteriological studies using the method of separated impedance". This device is highly productive as it examined 64 samples at the same time. Impedance microbiology is an indirect cultural method for detecting microorganisms using the measurement of electrical resistance (impedance) of cells. The express analyzer BACTrac 4300 registered changes in the electrical resistance of the nutrient medium which occurs under the influence of the growth and life processes of microorganisms in the sample under study. The unique ability of a bacteriological analyzer is to measure two parameters, the electrode impedance (e-Parameter) and the impedance of the medium (m-Parameter) which allowed to conduct a wide range of qualitative and quantitative studies.

The measurement of the m-Parameter was a relative decrease in the impedance of the medium, expressed as a percentage of the initial measurement. m-Parameter basically reflected that part of the impedance that is associated with the active conductivity. It was directly influenced by the ionic composition of the medium with the growth of microorganisms as well as the sample used for analysis. For these reasons with a high electrical conductivity of the nutrient medium this parameter lost its significance. The composition of the nutrient medium did not influence the value of the e-Parameter which is very important in cases when media with high salt content are used. Therefore, e-Parameter was chosen for the study. When the specified threshold values for the e-Parameter were exceeded the sample was evaluated by the traffic light system-red, yellow, green (if time intervals were specified) and/or there was an automatic counting of microorganisms in the sample under investigation (in case of using a calibration file).

The sanitary state of the soil was estimated from the total number of mesophilic aerobic and facultative anaerobic microorganisms (QMAFAnM, standard plate) in 1 cm (mL) or 1 g of substrate. The total number of microorganisms was determined on a BiMedia 001B nutrient medium (manufactured by HiMedia) at a temperature of 30°C.

Sanitary-indicative bacteria of *Escherichia coli* (*Escherichia, Shigella, Salmonella, Citrobacter*) were cultivated on enrichment medium buffered peptone water, *Listeria* on nutrient broth to isolate and cultivate *Listeria* for 24 h, determine contamination indices then were re-inoculated into BiMedia nutrients 205A, 401 A, XLD (Xylose-Lysine-Desoxycholate agar), Ploskirev, Kligler, Simmons and Christensen to identify generic and species affiliation. All crops were cultivated at a temperature of 37°C. The soil was assessed as "Clean" without any restrictions on sanitary-bacteriological parameters in the absence of pathogenic bacteria and the index of sanitary-indicative microorganisms up to 10 cells/g of soil (Sanitary rules and regulations, SanPin 2.1.7.1287-03).

The results are statistically processed. The software on the microbiological analyzer BACTrac is represented by: MicroTrac a program that manages data, monitors the measurement and obtains the results of analysis and MicroAssist a program for further work with the measurement results.

RESULTS AND DISCUSSION

The standard plate (QMAFAnM) index is an integrated indicator presented by various taxonomic groups of microorganisms which gives an idea of the epidemic situation in the region and the processes of self-purification of the biotope. The standard plate (QMAFAnM) is the most common test for microbial safety. This is based on the assumption that the more an object is contaminated with organic substances, the higher is the standard plate (QMAFAnM) and the more likely is the presence of pathogens. However, high values of the index can be due to saprophytes and pathogens will be absent. Therefore, it is more appropriate to regard the total number of microorganisms as an indicator of the intensity of environmental pollution by organic substances. The results of the study are presented in Table 1.

In the course of the work, it was established that environmentally friendly soil in terms of the number of microorganisms contained in it was taken in the area of the regional clinical Hospital No. 1-OKB No. 1, the regional blood transfusion station and a children's play ground. It was established that during the construction of these facilities it was completely replaced. Relatively high rates of microbial contamination were detected in samples on the territory of the McDonald's fast food supermarket which indicated a zone of increased risk and a high rate of multiplication of microorganisms (Kondakova, 2005; Gromov, 2010; Kamysheva, 2016 and Labinskaya, 2004). The quantitative composition of microbes in other areas under study is approximately the same and did not exceed the permissible limits.

Given that soil is a factor in the transmission of intestinal infections, direct detection of pathogenic

Table 1: Total number of mesophilic aerobic and facultative anaerobic microorganisms (standard plate)

Sample No.	Readings of the instrument	The standard plate (QMAFAnM) per 1 g of soil
1	5.0E+2 CFU	$5.0\times10^{2}\times10 = 5.0\times10^{}$
2	9.3E+2 CFU*	$9.3\times10^{3}\times10 = 9.3\times10^{3}$
3	4.3E+2 CFU	$4.3\times10^{2}\times10 = 4{,}3\times10^{3}$
4	9.4E+2 CFU*	$9.4\times10^{3}\times10 = 9.4\times10^{3}$
5	3.6E+2 CFU	$3.6\times10^{2}\times10 = 3.6\times10^{3}$
6	1.3E+5 CFU*	$1.3\times10^{5}\times10 = 1.3\times10^{6}$*
7	1.9E+3 CFU	$1.9\times10^{3}\times10 = 1.9\times10^{4}$
8	1.2E+3 CFU	$1.2\times10^{3}\times10 = 1.2\times10^{4}$
9	1.8E+3 CFU	$1.8\times10^{3}\times10 = 1.8\times10^{4}$

* -p<0.05; e-Parameter is the impedance of the electrode; CFU-the number of Colony Forming Units

Table 2: The cultural and biochemical properties of bacteria

Sample No.	Growth on Ploskirev's medium	Growth in the XLD medium	Citrate	Lactose	H_2S	Urea	Type of microorganism
1	-	-	+	+	-	-	*Citrobacter diversus*
2	-	-	+	+	+	-	*Citrobacter freundii*
3	Pink colonies	Yellow colonies	-	+	-	-	*Escherichia coli*
4	-	-	+	+	-	-	*Citrobacter diversus*
5	Pink colonies	Yellow colonies	+	+	-	-	*Citrobacter diversus*
6	-	-	+	+	+	-	*Citrobacter freundii*
7	Gray colonies	Yellow colonies	+	+	-	-	*Citrobacter diversus*
8	Pink colonies	Yellow colonies					*Citrobacter freundii*
9	Gray colonies	Yellow colonies					*Citrobacter diversus*

"+" The presence of a characteristic; "-" Absence of a characteristic

microbes in the biotope is carried out only when investigating outbreaks of infectious diseases. As indirect indicators are data of the fecal contamination of the object. In the course of the study, the intensity of the biological load on the soil in different parts of Tyumen was established. In all studied samples, bacteria of the *Escherichia coli* group were found. Identification of enterobacteria by biochemical activity was carried out on differential diagnostic environments. On the environment of Kligler, the bacterial saccharolytic activity was evaluated their ability to break down lactose and proteolytic activity their ability to decompose the protein to form hydrogen sulphide. On the simmons medium, germs grew using citrate as the sole source of carbon. Microorganisms that break down urea, alkalinized the medium of Christensen as a result of which it was colored. The presence in the soil of coliform bacteria which do not possess oxidase activity and ferment lactose with the formation of acid and gas may indicate fresh fecal contamination of the object (Table 2).

Thus, in the samples taken from the playground, the square of the 400th anniversary of Tyumen, the territory of school No. 29, the bacteria *Citrobacter diversus (C. diversus) Citrobacter freundii (C. freundii)* were identified. Gram-negative bacteria with rounded ends were found in smear samples. In the soils of the regional clinical hospital No. 2-OKB No. 2, the regional blood transfusion station, the McDonald's Fast Food Supermarket, *Alexander Moiseenko* garden square there were found: 1ac+, lac- *Escherichia coli (E. coli), C. freundii, C. diversus. Salmonella* is not detected.

It is known that the soil is the source of sapronotic infections. A characteristic feature of causative agents of saprozoonoses is their ability to remain in the interepidemic period in environmental objects (Kamysheva, 2016). In this connection, it is important to investigate the relationship between *Listeria* and bacilli when they inhabit the soil with a saprophyte microbiota which is of great ecological and epidemiological importance. By the method of separated impedance no *Listeria* were found in the samples under study. Found in all samples there are spore-forming, catalase negative bacteria bacillus cereus. The latter cause in people gastric diseases as well as septicemia, endocarditis, the central nervous system damage. For these reasons, the control of the named objects on the content of bacteria with application of reliable accelerated methods of testing becomes more and more actual.

Consequently, in the central part of the city with a high population density, the biological load on the soil is very high and as a consequence, the indices of sanitary-indicatory microorganisms are high which along with sanitary and chemical indices, indicates an ill-being and an increased risk of infection. It is proved that the soil of hospital establishments and public food service enterprises has the maximum degree of epidemiological danger ($p<0.05$). The permissible level of contamination is established at sites in areas of the city remote from the center.

CONCLUSION

The proposed method based on the divided impedance in comparison with the classical methods of microbiological research makes it possible to obtain fast (for several hours) and reliable results for a large number of simultaneously investigated samples. And also an undoubted advantage of this device is its portability and compactness. The classical methods of microbiological research used in the practice of bacteriological laboratories, require a great deal of labor and time.

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