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POLIO VIRUS INFILTRATION DEEP INTO SOILS FERTILISED WITH MUNICIPAL SEWAGE IN FIELD CONDITIONS

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ABSTRACT

The research aimed at defining the migration potential for the polio enterovirus in two soil profiles (podzolic soil and black earth) fertilised with sewage. The experimental fields were being fertilised with a 1:1 mixture of effluent and atenuated polio virus, strain Lsc2ab. After 28 days samples were taken for virus-oriented research. A slight virus penetration deep into the soil profiles was observed. The highest titres were obtained in the surface layer (0-2.5 cm) and they were, respectively: for the podzolic soil - $10^{5.72}$ $TCID_{50}$/g and for black earth $10^{4.33}$

$TCID_{50}$/g of the soil. The polio virus did not migrate beyond the humus layer. In podzolic soil it was observed to 20-25 cm deep, however in the black earth 15-20 cm deep. The physical properties and chemical analysis of the podzolic soil were slightly more favourable for virus migration deep into the soil.

Key words: polio virus, municipal sewage, infiltration, podzolic soil, black earth

INTRODUCTION

Municipal sewage effluent management, because of its micro-biological contamination, remains a significant hygiene concern. It may contain numerous pathogenic microbes as well as eggs and oocysts of the parasite [15]. Specially significant, for epidemic and epizootic reasons, are the viruses present in sewage. A constant development of research methods allowed to isolate over 130 virus types from sewage [8]. Most frequently adenoviruses are observed as well as polio viruses and Coxsack viruses A and B, ECHO-viruses and contagious hepatitis virus [7]. One shall stress that sewage treatment process, with the technologies applied so far, does not cause a complete elimina-tion of pathogenic viruses [15].

Viruses, along with organic fertilisers penetrate into the environment. Some of them undergo inactivation, however the remaining ones may be adsorbed on soil particles and, consequently, transported into its deeper layers [16].

The research aimed at defining the behaviour of polio enterovirus in two different soil profiles fertilised with municipal sewage.

MATERIALS AND METHODS

The research was conducted in the podzolic soil and black earth. At the first stage of the experiment, physical properties and chemical analysis of the selected soils were defined e.g. pH, cation exchan-geable capacity and organic matter content, soil pore volume distribution, grain composition [10,11]. Also the chemical analysis of the municipal sewage applied for the experiment was examined. Dry matter, basic minerals (nitrogen, phosphorus, potassium, calcium) as well as the reaction and heavy metal content were defined [13].

The enterovirus penetration deep into the soils was observed in two podzolic soil and black earth experimental fields. The field size was defined with metal pipes, 23 cm in diameter. The polio virus with the titre of 1.5 x $10^{8.0}$ $TCID_{50}$/ml was applied for the research. The Lsc2ab polio virus was being multiplied on cell line Hep 2 applying the Eagle minimal essential medium with antibiotics added [9].

The podzolic soil field was fertilised with 1306 ml of the 1:1 mixture of virus and municipal sewage, whereas the black earth was fertilised with 1450 ml. With a weekly break the fields were watered; the amount of water equalled the average rainfall in the region. The test was conducted in autumn, and it took into consideration environmental conditions (temperature, rainfall, sun exposure time). After 28 days samples were taken with sterile spoons every 2.5 cm up to 15 cm deep, every 5 cm up to 30 cm deep and every 10 cm deeper. The soil samples were frozen which was repeated 3 and 2 times. 10% fetal serum was applied for the enterovirus elution [9]. The virus infectious titres were defined with the generally applied method in cell lines on micro-plates. The titres were defined with Kärber's method and they were presented as the titres of $TCID_{50}$/g of the soil [18].

RESULTS

The research results of physical properties and chemical analysis of the soils are presented in Table 1. The podzolic soil was homogenous in reference to grain size distribution. It consisted mostly of quartz sands and thick dust with very small sorption capacity. The humus layer, poor in organic matter, had a slightly acid reaction, however the parent rock - acid. A high number of micro-pores determined good drainage potential of the podzolic soil. The other of the soils researched, black earth had the grain size distribution of the strong muddy sand. Due to its high organic matter content (7.9 %), it had a very high cation sorption capacity (Tab.1).

Table 1. Physical properties and chemical analysis of the soils researched

Genetic layer	Layer cm	Fraction content in mm (%)					pH in KCl	Percentage of macro-pore volume in diameter (μ m)					Cation exchange-able capacity me/100 g	Organic matter %
		1.0-0.1	0.1-0.05	0.02-0.002	< 0.002	< 0.02		Macro-pores		Mezo-pores		Micro-pores		
								>300	300-30	30-5	5-0.2	<0.2		
Podzolic soil – profile 1 –														
Ap	0-30	74	18	4	1	5	6.7	3.3	25.3	6.8	6.3	2.3	7.0	1.7
C	> 30	71	24	2	2	4	4.4	4.1	27.5	5.5	3.7	1.8	2.92	-
Black earth – profile 2														
A	0-45	68	12	8	5	13	7.6	3.7	11.1	9.1	8.6	12.1	26.28	7.9
Ccagg	> 45	45	10	16	21	37	8.2	3.4	4.6	3.7	4.2	21.9	30.64	-

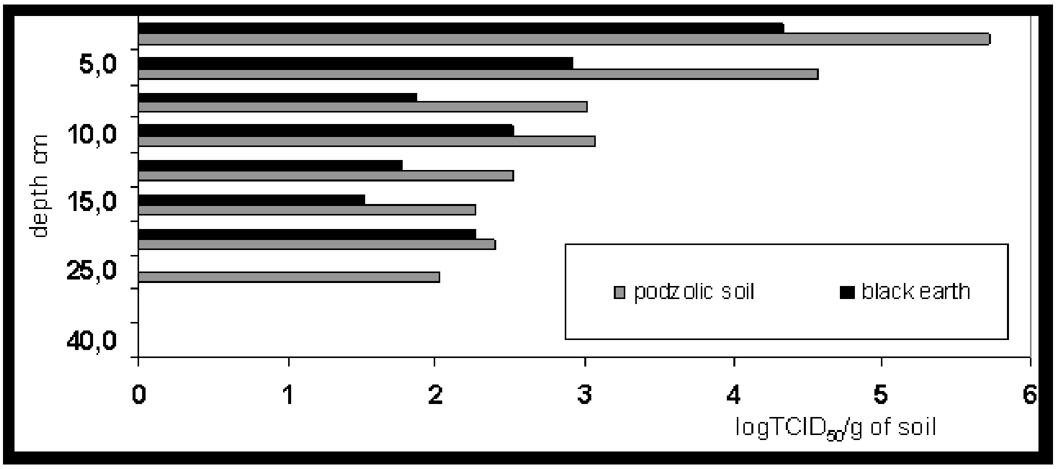
The reaction of that soil differed considerably from the one of the podzolic soil as due to a considerable content of carbonates, pH of the humus layer amounted to 7.6, however the pH of the mother rock - 8.2. Besides a considerably lower number of macro-pores created worse drainage conditions when compared to the other soil. The research included air temperature control, which fluctuated between 3.3-8.1°C. The mean soil temperature fluctuated from 3.4-9.4°C 5 cm deep, 3.4-9.7°C 10 cm deep and 4.0-10.4°C 20 cm deep. During the field experiment the total rainfall observed amounted to 15.2 mm and the sun exposure time amounted to 3.7 to 5.1 hours per every 24 hours. The chemical analysis of the municipal sewage applied to fertilise soils is presented in Table 2. A low dry matter content (1738 mg/dm^3) as well as a low organic content were observed. The sewage reaction was 7.3.

Table 2. Municipal sewage chemical analysis

Feature	Dry matter content mg/dm^3	Post-roasting residue mg/dm^3	Post-roasting loss mg/dm^3	Content				Metal content in mg/dm^3				pH in KCl
				nitrogen mg N/dm^3	phosphorus mg P/dm^3	potassium mg K/dm^3	calcium mg Ca/dm^3	cadmium	lead	zinc	copper	
Municipal sewage	1738	654	1084	102	187	52	95	1.1	28.2	71.6	18.4	7.3

The polio virus behaviour in soils fertilised with municipal sewage is presented in Figure 1. During the field experiment, the natural soil structure was maintained with a slight virus penetration deep into the soil profiles. In podzolic soil the polio virus was isolated up to 20-25 cm deep. The highest titre was observed in the surface layer of the soil (0-2.5 cm) and it fluctuated between $10^{5.27}$ - $10^{6.27}$ $TCID_{50}$/g of the soil. With depth the virus titre decreased to $10^{2.02}$ $TCID_{50}$/g of the soil 20-25 cm deep (from $10^{1.77}$ - $10^{2.27}$ $TCID_{50}$/g of the soil). The polio virus in the black earth migrated to 15-20 cm deep with the mean titre of $10^{2.27}$ $TCID_{50}$/g of the soil. One shall take into consideration that the high polio virus titre observed in the surface layer (0-2.5 cm) of the soil, $10^{4.33}$ $TCID_{50}$/g on average, was decre-asing considerably to $10^{1.87}$ $TCID_{50}$/g of the soil 5.0-7.5 cm deep. The decrease in virus titre with depth was more rapid than in podzolic soil. With consequent depths researched, no systematic decrease in virus titres was observed (Fig. 1).

Fig. 1. The infiltration of the polio virus suspended in the sewage deep into the soils researched



DISCUSSION

In the experiment conducted the viruses migrated up to 15-20 cm deep in the black earth and 20-25 cm deep in the podzolic soil with the highest titre observed in the soil surface layers (Fig. 1). The virus retention in the soil upper layer, observed in the present research, is present also in the papers of other authors. In their laboratory research, Filip at al. [4] observed the retention of 99 % viruses in the 5 cm surface layer of the soil column, whereas Reddy at al. [14] in 2 cm layer. Wellings at al. [17] in forest soils (sandy) observed 7 m. deep virus penetration after 28 days.

The research of physical properties and chemical analysis of the soil showed that due to the grain size distribution and the low sorption capacity, the podzolic soil appeared more favourable for virus migration (Tab.1). However the polio virus penetration depth difference in the black soil was smaller than expected. Undoubtedly an important factor which limited virus migration potential in the soil was their adsorption on soil particles. It seems that the low pH of the podzolic soil enhancing virus adsorption in the field environment was powerful enough to facilitate greater filtration due to the number of the macro-pores. Dizer [3] observes that the impact of the pH on virus adsorption processes in sand remains higher than in the other soils, whereas the maximum adsorption takes place with pH 5.1. It seems from the literature that sewage fertilisation enhances adsorption proper-ties of sandy soils proportionally to the cation volume provided with the sewage [17]. On the other hand, the presence of mega-pores in the browned black earth, which can be the place of a quick microbe transport deep into the soil [6], could have limited a negative influence of sorption complex and a bigger amount of organic matter on the transport processes. Due to numerous processes taking place in the soil simultaneously (virus adsorption with a loss or main-taining of activity, desorption, migration deep into the soils) it is difficult to define which of them in at given time is most intensive. Besides viruses may behave in a way which is not typical [1].

In black earth lower polio virus titres were observed at respective depths. On the one hand it could have been due to a stronger virus adsorption in the soil with a higher organic matter content and a high sorption complex; on the other hand a negative impact of the indigenous soil micro-flora [16, 5]. The sandy soils (podzolic), with a scarce amount of organic matter, are considered poorer in indigenous flora when compared to the black earth, which limits the negative influence of bacterial micro-organisms on enteroviruses. The research by Deng and Cliver [2] provided more evidence that the polio virus is sensitive to the proteolitic enzymes produced by some micro-organisms.

The fluctuations in virus titres observed, especially in the black earth, may signify virus desorption processes in the soil. Desorption processes depend on the characteristics of virus species and here one has to mention that the polio virus elution (Lsc2ab) from the soil is mostly poor [12]. Although the polio virus in the present research did not penetrate beyond the humus layer, the fact that high titres in the surface layer are observed remains disturbing as plant contamination may pose an epidemic and epizootic hazard. One cannot also eliminate the virus migration deeper into the soil layers with more intensive rainfalls.

CONCLUSIONS

1. In the field environment the polio virus did not penetrate beyond the humus layer of the soils researched.
2. The highest enterovirus titres were observed in the soil surface layers (0-2.5 cm).
3. High enterovirus titres observed in the soil surface layers may pose a hazard of plant contamination and an epizootic and epidemic threat.
4. It was observed that the podzolic soil due to its physical properties and chemical analysis facilitated a deeper polio virus penetration.

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