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Effectiveness of different means of disinfection against soil foci of anthrax (*Bacillus anthracis*) burials at a depth of up to 3.5 m: an experimental study

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ABSTRACT

There are over 2,000 anthrax-infected soil foci in Kazakhstan, posing a potential threat of infection with anthrax to the population. The anthrax soil foci are found in all regions of Kazakhstan, often located near residential buildings, interfering with the promising development of the region. Experimental work was carried out on the development of disinfection of the deep soil layers at a depth of up to 3.5 m using the drilling method. The experiments were carried out on a modular site measuring 210 cm by 280 cm with a natural occurrence of soil. The experimental site disinfection method included the complete filling of 12 prepared wells with BA-12 disinfectant. The soil in the wells had been previously contaminated with a vaccine culture of anthrax (*Bacillus anthracis*) pathogen 55-VNIIVViM. The total volume of the disinfectant solution required for complete disinfection of the entire volume of the soil (the experimental site, amounted to 1,635 L) was determined. According to the authors, the main practical conclusion of the study was the possibility to use the scheme of disinfection of soil foci of anthrax burials developed during the experiment for carrying out production and construction work at the site of existing burials.

Key words: Drilling method, Formaldehyde, Morbidity, Soil.
Article type: Research Article.

INTRODUCTION

Parasitic contamination of the soil-by-soil infection pathogens occurs all over the planet (Khutoryanina *et al.* 2021), however, it is characterized by focal character, i.e. limited distribution in a certain area (Gavrilov *et al.* 2011; Sajjala *et al.* 2019; Khezri *et al.* 2021; Abed Almjalawi *et al.* 2022; AL-Lami & Al-Mayaly 2022; Javed *et al.* 2023; Shikverdiev *et al.* 2023). In this case, the soil functions not as a passive repository of soil infection pathogens, but as a place where, under certain conditions, the parasitic agent repeatedly performs a full cycle of its development, preserving its viability (Yang *et al.* 2021; Mukhamadiyev *et al.* 2023; Tarigan *et al.* 2023). Based on the focus of our study, we emphasize that the soil is an incubation zone and concentration of anthrax, *Bacillus anthracis* (Driciru *et al.* 2020; Liddington 2021). The anthrax soil foci can pose a potential threat in case of occurrence in the territory with a natural disaster focus (collapse of riverbanks and ravines, swamping, floods, etc.) where construction work or mining operations are carried out (Goryacheva *et al.* 2016; Simonova 2016; Popova *et al.* 2017). However, based on the topic of our study, it is necessary to pay attention to the fact that soil foci may be in the zone of more intensive agriculture (Yessimbek *et al.* 2022) which poses an additional danger for anthrax infection in humans and animals (Simonova *et al.* 2013; Kartavaya *et al.* 2016; Marinin *et al.* 2017). Despite the relatively low incidence of anthrax in recent decades, the risk of complications of the situation persists (Centers for Disease Control & Prevention 2006, 2010; Berger *et al.* 2014). The term during which the anthrax pathogen is preserved in the soil is not defined, depending on the type of soil, conditions, and pH (Eremenko *et*

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al. 2017; Yamtitina & Makarov 2018). For example, the largest outbreak of anthrax occurred in Yamal, several decades after the last manifestation of the activity of the soil foci located in this territory (Selyaninov *et al.* 2016; Kletsko *et al.* 2018; Plotnikov & Glazunova 2019). According to the authors (Gavrilov *et al.* 2011; Shikverdiev *et al.* 2023), the current situation, considering the content of anthrax animal burial grounds in the regions of the Russian Federation that are unsafe regarding this disease, is a time bomb. We established that the anthrax soil foci are found in several regions of the Republic of Kazakhstan. Such foci are located in the Turkestan region (Turkestan, Kentau), in the Akmola region (Arshaly), in the Zhambyl region (Kurenbel and Koshkarata villages), and Aktobe. Potential threats of anthrax outbreaks reduce the efficiency of urban development (Balova *et al.* 2021; Ospangaliyev *et al.* 2022) and villages as well as the entail medical (Mokhov *et al.* 2022), social (Yessimbek *et al.* 2022), and economic (González 2020) issues for public health and human life (Klimovskikh *et al.* 2023). Solving these problems is a difficult task, in particular, due to the need, on the one hand, for the construction of residential neighborhoods (Mayboroda & Spirin 2023) with schools and water supply, and on the other hand, development plans affect the lands located on the territory of sanitary protection zones (Rodnyansky *et al.* 2021; Mukhamadiyev *et al.* 2023) of anthrax burials. Therefore, there is a need for research to find effective methods used to disinfect the soils of anthrax burials, considering the capabilities and resources of a particular region.

Methods used for soil disinfection in anthrax burials

According to numerous data, most of the disinfectants tested at different times in the form of solutions, when applied to the soil, bind to its components and become inactive (neutralized), without ensuring the death of not only spore-forming microorganisms but even asporogenic pathogenic microflora and viruses (Polyakov 1975). Therefore, an important and urgent problem in medicine and veterinary medicine nowadays is the development of a highly effective disinfectant and disinfection method designed for the accelerated and reliable elimination of soil foci containing pathogens (including anthrax), resulting from burial, slaughter, or death of sick animals, or as a result of man-made accidents and disasters or bioterrorist attacks (Goryacheva *et al.* 2016; Schmid & Kaufmann 2022). Over the past 50 years, various methods have been proposed for the elimination (relief) of soil foci, from the transfer of animal burial grounds to places inaccessible to humans and animals and concreting the burials with concrete, including directly at the place where the carcasses are buried, to the sanitation of the soil of the animal burial ground by chemical or physical methods. In the period from 1970 to 1979, a radical method of soil disinfection for anthrax cattle burial grounds, including old ones, at a depth of up to 2 m with a mixture of ethylene oxide and metal bromide under a sealing coating of polyamide film was developed and introduced into veterinary practice (at that time, the PK-4 brand, manufactured by the USSR, was used). The gas method provides disinfection of the soil to a depth of at least 2 m directly in the places of treatment, i.e., without soil excavation and movement. The possibility of disinfecting anthrax animal burial grounds using the gas method is still being considered by some scientists (Sokolova, 2013; Popov & Volkovskii 2016). However, this method of disinfection is difficult to perform and requires additional expensive equipment, as well as special liquefied gas (for example, a gas mixture of ethylene oxide with methyl bromide). In addition, at any stage of the work on soil disinfection, there is a high risk of emergencies in the form of leakage or release of toxic gases into the atmosphere. Research on the development of effective disinfectants was carried out by scientists in the Russian Federation. Thus, Gerasimov *et al.* (2021) proposed a disinfectant which contains a solution of hydrogen peroxide, peracetic acid, isopropyl alcohol, and water. The claimed solution is characterized by high stability and lack of toxicity to animals and plants. Microbiological studies have established that, after disinfection treatment of loamy and podzolic soil, there are no viable anthrax spores in all samples taken from different soil depths up to 2,000 mm in the model of the anthrax burial ground. The chemical-analytical studies of the spent disinfectant solution showed that the activity of the agent decreased by no more than 60% of the initial activity (Gerasimov *et al.* 2021). A team of researchers led by Wood (2021) conducted studies to evaluate soil disinfection using the method of dry heat treatment. The study was conducted in two stages using loamy, clay, and sandy soils, as well as biological indicators contaminated with spores. The initial experiments were carried out in a climatic test chamber with a controlled temperature from 80 to 110 °C, with or without the addition of moisture, and with the time of contact from 4 hours to 7 weeks. The tests were then expanded to assess the thermal inactivation of spores in small soil columns where a heating plate set at 141°C was applied to the soil surface. These column tests were carried out to determine the time required for the inactivation of spores depending on the depth and type of soil. The results of the initial stage of the tests showed that an increase in temperature and relative humidity reduced the time

required to obtain samples in which no bacterial spores were detected. For the test at 80 °C without adding moisture, it took 49 days to obtain soil samples without spores found in clay and loam. At a temperature of 110°C, it took 24 hours to obtain samples where no spores were detected. In column tests, spores were not detected at a depth of 2.5 cm after four days and at a depth of 5.1 cm after 21 days for two of the three soil types. The experiments described in the study demonstrate the possibility of using dry thermal methods for the disinfection of various soil types that have been superficially contaminated with the anthrax pathogen. Another group of scientists (Richter *et al.* 2022) conducted studies on the comparative evaluation of the effectiveness of decontamination using liquid formaldehyde solutions for three soil types (sand, loam, and clay) against the *B. anthracis* and *B. atrophaeus* spores. During the experiments, each soil sample was contaminated with pathogen spores at a concentration of 1×10^8 colony-forming units (CFU). During the experiments, two concentrations and two volumes of a liquid formaldehyde solution were added to the soil samples and left in contact for 24 or 48 hours. The disinfection efficiency was evaluated at 22 °C or 10°C with or without lids on sample jars. Complete inactivation (with no spores isolated from soil samples, which usually provides > 7-fold logarithmic reduction) of *B. anthracis* occurred in all soil types in five of the six tests, while complete inactivation of *B. atrophaeus* was achieved in all soil types for three of the six tests. The results showed a higher probability of complete inactivation of spores for samples that received a higher volume of formaldehyde. In general, the use of a liquid formaldehyde solution (2.5-5%) was highly effective in inactivating entire populations of spores (usually > 107 CFU), both for *B. anthracis* and *B. atrophaeus* studied in soil matrices. Covering the soil after application will allow using less formaldehyde solution without affecting the overall efficiency of the process. Although studies in this field have been conducted since the middle of the 20th century, there is a relatively small selection of means for carrying out disinfection work on the destruction of *B. atrophaeus* pathogens in the external environment. Therefore, the purpose of our study was to search for optimal methods of the soil foci disinfection to a depth of 3.5 m. using universal economical preparations exhibiting the bactericidal and sporicidal properties and are available for intensive use (to cover large areas of Kazakhstan). Further, the study presents the results of an experimental study on the development of a soil foci disinfection scheme.

MATERIALS AND METHODS

The experimental study was conducted in 2022 at sites in Turkestan (Kazakhstan) and the laboratory of the Kazakh Scientific Research Veterinary Institute. The study consisted of two main stages (soil disinfection and soil sampling, followed by its analysis in the laboratory). Each of the main stages was divided into additional stages.

Soil disinfection

Experimental methods of deep soil layer disinfection were tested using the drilling method. In experimental work, a special drilling rig TS 30 was used to work with rock strength categories up to 10, with a maximum initial bore bit diameter of 150 mm, with a rotational speed of 1,680 rpm. Three experimental sites measuring 210.0 cm by 280.0 cm^3, i.e., similar in size to the anthrax burial site located in Turkestan, and in need of urgent disinfection and liquidation, were prepared for experiments at the site. At all three experimental sites, 12 wells were drilled using a drilling rig at a distance of 50 cm from each other and to a depth of 3.0 m (Fig. 1). Earthen sides were made along the perimeter of each section to exclude the spreading of solutions. To simulate soil contamination, tap water with spores of the vaccine strain of the anthrax pathogen *B. a.* 55-VNIIVViM was previously poured into all prepared wells to a depth of 3.0 m at a rate of 3.0 L per well, and the concentration of the solution was 1.0 $\times 10.^8$ CFU cm^{-3}. The prepared sites were left for 24 hours for the complete distribution of the vaccine culture in the depth of all wells and on the surface of the site. After 24 hours, at two sites (the experimental and the first control site), all prepared pits and the surface were filled with disinfectant solutions. At the first experimental site, a 20% solution of the BA-12 disinfectant (experimental site) was used. Registration certificate No. RK-VP 5-4305-20 was received for the specified disinfectant. It is also included in the register of registered medicines for use in veterinary medicine of the Republic of Kazakhstan. Veterinary disinfectant BA-12 is intended for preventive and forced disinfection of veterinary facilities and soil, consisting of the basic and buffer solutions. The composition of the basic solution includes: dodecyl-dimethyl-ammonium chloride (6.83%), dodecyl-dimethyl-ammonium bromide (12.5%), glutaraldehyde (11.0%), isopropyl alcohol (40.5%) and purified water (up to 100%). The composition of the buffer solution includes: carbamide (50%), isopropyl alcohol (6.5%), and purified water (up to 100%).

Fig. 1. Drilling of control wells after a single treatment.

The wells and the surface of the second site were filled with soda ash solution (the first control site). At the third site, disinfectant solutions were not used, and the wells were filled with ordinary tap water (the second control site). We tried to fill the wells at each site in a short period (3-4 min), i.e., almost simultaneously. The wells were treated at the experimental sites three times, every 24 h. The process of selecting control samples after double treatment was difficult, since the soil was moist at a depth of 0.8 m to 3.0 m (Fig. 2). After the third treatment of the sites, control sampling was even more time-consuming due to the complete moistening of the soils to the full depth, since the soil in the wells and on the surface was completely wet and the auger of the drilling machine rose with delays. 12 hours after each treatment, control soil samples were taken from different horizons at all three sites. Samples were taken both from treated wells and wells drilled between two adjacent ones (control intermediate ones), as well as to the side at various distances from the end well. Disinfectant solutions were prepared immediately before use. Thus, the working solution of the BA-12 disinfectant was prepared by mixing the basic and buffer solutions with the addition of ordinary water to them. For the experiment, a 20% concentration of the preparation was used. For the second site, a freshly prepared hot 10% NaOH solution was used.

Fig. 2. The surface of the experimental area after double humidification.

The prepared wells and the surface at the experimental and two control sites were treated with solutions three times, with an interval of 24 hours. To exclude the process of evaporation of the disinfectant solutions and water, all the surfaces of the treated soil were covered with a polyethylene film, which was fixed along the perimeter of the site with 15-cm high bulk land sides. The soil temperature was 15 °C. Soil samples were taken from the experimental and control sites to assess the disinfecting effect of the solutions.

Selection and Examination of Soil Samples

Sampling was carried out both from the surface of the sites and different depths of wells, namely: 3.5 m — 3 m — 2.8 m — 2.5 m — 2.2 m — 2.0 m — 1.8 m — 1.5 m — 1.3 m — 1.0 m — 0.8 m — 0.6 m — 0.4 m — 0.2 m

— 0.1 m. In addition, to control the horizontal dissociation of disinfectant solutions, intermediate pits were drilled exactly in the middle between the experimental wells, as well as away from the end wells at a distance of 20 cm — 30 cm — 40 cm, and 50 cm. These wells were control wells to assess the complete disinfection of the entire soil of the sites. Soil samples from both experimental and control (intermediate) wells were taken every 12 hours from the moment of their initial treatment. During the experiment, all soil samples were neutralized with a universal neutralizer. To determine the presence or absence of *B. anthracis* vaccine culture, as well as other microorganisms, the experimental and control soil samples were packed in plastic containers and delivered to the laboratory of the Institute for further examinations. In the bacteriology laboratory of the Institute, all delivered samples were examined using the methods of indication and identification of the *B. anthracis* pathogen. Initially, samples were prepared for examination, which included the extraction of soil samples in a sterile saline solution. The obtained soil extracts were examined under a microscope and seeded on the dense and liquid nutrient media. Identification of the obtained isolates was carried out according to the following tests: morphology of the microbe in Gram-stained smears, growth in broth and Hottinger agar; tests for hemolytic and lecithinase activity; sensitivity to penicillin and a sample with anthrax bacteriophage. The effectiveness of disinfectant solutions was assessed by the presence or absence of growth of microorganisms, including the vaccine strain *B. a.* 55-VNIIVViM. After carrying out all the experimental work, the second and third sites were treated three times with a disinfectant solution, and the laboratory negative results were obtained when checking soil samples for the presence of microorganisms.

RESULTS

During the experiment, it was found that at the first filling, 39.0 l of solution was needed to fill each pit. To fully moisten the surface on the experimental site, 105.0 L of the solution was used. A total of 573 L of solution were used for a single treatment, including 468 L for 12 pits and 105 L for moistening the soil surface. In total, 1635 L solution and water were used for the three-time treatments of the experimental and two control sites, respectively. During the experiments, it was found that at the first site, after 12 hours between two neighboring wells, the soil was moist at a depth of 1.2 and up to 3.5 m, as well as 10 cm away from the end wells, at similar horizons. After secondary filling between two adjacent pits, complete horizontal dissociation of the BA-12 solution was noted on all horizons, and from the end well the solution spread 30 cm to the side. After filling the pits three times, the soil between the neighboring wells was already completely wet, and the solution was found at a distance of 35 cm away from the end wells. Thus, when using the BA-12 disinfectant with double treatments, complete horizontal dissociation of the solution was observed at a distance of 25 cm to 35 cm. At the second site, where the soil was treated with a solution of soda ash, the dissociation of the solution between two adjacent wells was not noted even after the third filling. Control wells showed that the solution spread from each pit 15-17 cm to the side, which did not allow for treating the entire volume of the site's soil. Similar results were obtained when controlling the horizontal distribution of ordinary tap water. Laboratory examination of delivered soil samples taken at regular intervals (every 12 hours) and from different depths showed mixed results. Thus, when examining soil samples from the site treated with BA-12 disinfectant, the absence of growth of microorganisms from soil samples taken from experimental (drilled) wells at a depth of 3.2 m to 3.5 m was noted after 12 hours. Cultures of microorganisms characteristic of ordinary soil were found in the soil from a level of 0.1 to 3.0 m, while no cells typical of anthrax culture were found. In this experiment, at the specified horizons (0.1 to 3.0 m), a decrease in the number of isolated cultures was found, compared to the control samples. Thus, in the latter samples, the presence of microorganisms was found in the range from 7.68 $\log_{10}$ CFU cm^{-3} (depth 0.1 m) and up to 1.86 $\log_{10}$ CFU cm^{-3} (depth 3.5 m). In experimental soil samples using the BA-12 solution, these indicators ranged from 5.21 $\log_{10}$ CFU cm^{-3} (depth 0.1 m) to 0.16 $\log_{10}$ CFU cm^{-3} (depth 3.0 m), while in deeper layers, i.e., 3.2 and 3.5 m, the presence of microorganisms was not established. When cultivating the soil with a 10% solution of soda ash, a drop in the number of microorganisms released was also observed. Thus, after treatment with this solution in soil samples from a depth of 0.1 m, the presence of culture was established, its concentration was 5.46 $\log_{10,}$ at a depth of 3.5 m and microorganisms were released in an amount equal to 0.06 $\log_{10}$. A comparative analysis of the effectiveness of both disinfecting solutions shows a declined number of microorganisms in experimental soil samples compared to the control samples. At an exposure of 12 hours, in the soil treated with a 20% solution of BA-12, the number of CFUs detected was significantly lower than when using a 10% solution of soda ash. Such a pattern was observed on all treated soil horizons, i.e., from 0.1 to 3.5 m. by an elevation in the exposure time to 24 and 48 hours, the

growth of microorganisms from the soil samples in the all horizons of the wells of the two experimental sites, including from the surface, was not established, i.e., all soil samples were sterile. The results of the study of soil samples treated with disinfectant solutions, as well as control samples are presented in Tables 1-3. It follows from the data in Table 1 that 12 hours after soil treatment, a dropped concentration of microorganisms was observed, and bacterial cultures were not isolated at all from samples taken from a depths of 3.2 and 3.5 m. When examining the soil from a site treated with 10% NaOH, 12 hours after application of the solution, the growth of microorganisms from soil samples from all horizons was established. However, after 24 hours and 48 hours of exposure, the growth in soil samples taken from all horizons of drilled wells was not detected (Table 2). In the soil samples obtained from the third site (the second control site), the growth of both aerobic and anaerobic microorganisms from all selected soil samples was observed. A gradual decline in the concentration of microorganisms was also observed in soil samples obtained from deeper layers. From the data given in Tables 1 and 2, it follows that a 20% solution of the BA-12 disinfectant and a 10% solution of soda ash have bactericidal and sporicidal properties when applied for 24 hours or more. By a 12-hour application of solutions to the soil, the growth of microorganisms was observed, except for two samples taken from a depth of 3.2 and 3.5 m, when they had been treated with BA-12 disinfectant. In both cases, a drop in the number of isolated cultures of microorganisms was found. Aerobic and anaerobic microorganisms were isolated in the control soil samples not treated with disinfectants throughout the entire period of the experiment. A comparative analyses of the presence of microorganisms in the soil after treatment with working solutions of disinfectants (BA-12 and NaOH), as well as in the control samples, by an exposure duration of 12 hours, are shown in Fig. 3.

Table 1. Results of microbiological studies of soil samples treated with a 20% solution of the BA-12 disinfecting agent.

Sampling depth, m	Presence of microorganisms after treatment, CFU/cm^3; After, hours					
	12 hours	24 hours	36 hours	48 hours	60 hours	72 hours
	BA-12 disinfectant (20%)					
0.1	5.23 $\log_{10}$	-	-	-	-	-
0.2	5.21 $\log_{10}$	-	-	-	-	-
0.4	4.83 $\log_{10}$	-	-	-	-	-
0.6	4.11 $\log_{10}$	-	-	-	-	-
0.8	3.91 $\log_{10}$	-	-	-	-	-
1.0	3.74 $\log_{10}$	-	-	-	-	-
1.3	3.25 $\log_{10}$	-	-	-	-	-
1.5	2.74 $\log_{10}$	-	-	-	-	-
1.8	2.28 $\log_{10}$	-	-	-	-	-
2.0	2.04 $\log_{10}$	-	-	-	-	-
2.2	1.96 $\log_{10}$	-	-	-	-	-
2.5	1.08 $\log_{10}$	-	-	-	-	-
2.8	0.46 $\log_{10}$	-	-	-	-	-
3.0	0.16 $\log_{10}$	-	-	-	-	-
3.2	-	-	-	-	-	-
3.5	-	-	-	-	-	-

Note: "-" means the absence of microbial growth in the sample.

DISCUSSION

Earlier experiments confirmed the high biocidal effectiveness of the BA-12 disinfectant (Ivanov *et al.* 2020). Therefore, the objective of our study was to test the solution in deep soil layers to the depth of the entire soil focus from 3.0 to 3.5 m. The experiments have shown that the drilling method, with the correct calculation of the number of wells, allows soil disinfection to the required depth. At 12 hours after applying the BA-12 disinfectant solution at a depth of 3.2 and 3.5 m, no microorganisms were detected, i.e., the soil treated with this preparation was sterile. When using a 10% solution of soda ash after 12 hours of exposure, growth in the cultures of microorganisms from all soil samples was noted. A longer exposure period of both disinfectant agents allowed negative results to be obtained from all experimental wells. From the soil of the control wells, the presence of diverse microflora was noted throughout the experiment. The obtained results can be explained by the presence of an additional buffer solution containing carbamide and isopropyl alcohol in the composition of the disinfectant. This solution exhibited a prolonged effect, reduced the neutralizing properties of the humus layer of the soil, and displayed conductive properties for the basic solution of the BA-12 disinfectant.

Table 2. Results of microbiological studies of soil samples treated with 10% soda ash solution.

Sampling depth, m	Presence of microorganisms after treatment, CFU/cm^3; After, hours					
	12 hours	24 hours	36 hours	48 hours	60 hours	72 hours
	Soda ash solution (10%)					
0.1	5.46 $\log_{10}$	-	-	-	-	-
0.2	5.24 $\log_{10}$	-	-	-	-	-
0.4	4.81 $\log_{10}$	-	-	-	-	-
0.6	4.14 $\log_{10}$	-	-	-	-	-
0.8	3.89 $\log_{10}$	-	-	-	-	-
1.0	3.72 $\log_{10}$	-	-	-	-	-
1.3	3.21 $\log_{10}$	-	-	-	-	-
1.5	2.69 $\log_{10}$	-	-	-	-	-
1.8	2.30 $\log_{10}$	-	-	-	-	-
2.0	2.12 $\log_{10}$	-	-	-	-	-
2.2	1.98 $\log_{10}$	-	-	-	-	-
2.5	1.14 $\log_{10}$	-	-	-	-	-
2.8	0.49 $\log_{10}$	-	-	-	-	-
3.0	0.20 $\log_{10}$	-	-	-	-	-
3.2	0.12 $\log_{10}$	-	-	-	-	-
3.5	0.06 $\log_{10}$	-	-	-	-	-

Note: "-" means the absence of microbial growth in the sample.

Table 3. Results of microbiological studies of soil samples not treated with disinfectants.

Sampling depth, m	Presence of microorganisms after treatment, after		
	12 hours	24 hours	72 hours
	Plain water		
0.1	7.68 $\log_{10}$	7.64 $\log_{10}$	7.63 $\log_{10}$
0.2	7.51 $\log_{10}$	7.49 $\log_{10}$	7.50 $\log_{10}$
0.4	7.10 $\log_{10}$	7.08 $\log_{10}$	7.11 $\log_{10}$
0.6	6.88 $\log_{10}$	6.89 $\log_{10}$	6.90 $\log_{10}$
0.8	6.67 $\log_{10}$	6.64 $\log_{10}$	6.65 $\log_{10}$
1.0	6.11 $\log_{10}$	6.14 $\log_{10}$	6.12 $\log_{10}$
1.3	5.96 $\log_{10}$	5.89 $\log_{10}$	5.89 $\log_{10}$
1.5	5.43 $\log_{10}$	5.57 $\log_{10}$	5.53 $\log_{10}$
1.8	4.98 $\log_{10}$	4.99 $\log_{10}$	4.97 $\log_{10}$
2.0	4.74 $\log_{10}$	4.80 $\log_{10}$	4.79 $\log_{10}$
2.2	4.42 $\log_{10}$	4.46 $\log_{10}$	4.44 $\log_{10}$
2.5	4.26 $\log_{10}$	4.24 $\log_{10}$	4.26 $\log_{10}$
2.8	3.85 $\log_{10}$	3.91 $\log_{10}$	3.89 $\log_{10}$
3.0	3.34 $\log_{10}$	3.38 $\log_{10}$	3.36$\log_{10}$
3.2	2.07 $\log_{10}$	2.12 $\log_{10}$	2.10 $\log_{10}$
3.5	1.86 $\log_{10}$	1.92 $\log_{10}$	1.90 $\log_{10}$

Note: "-" means the absence of microbial growth in the sample.

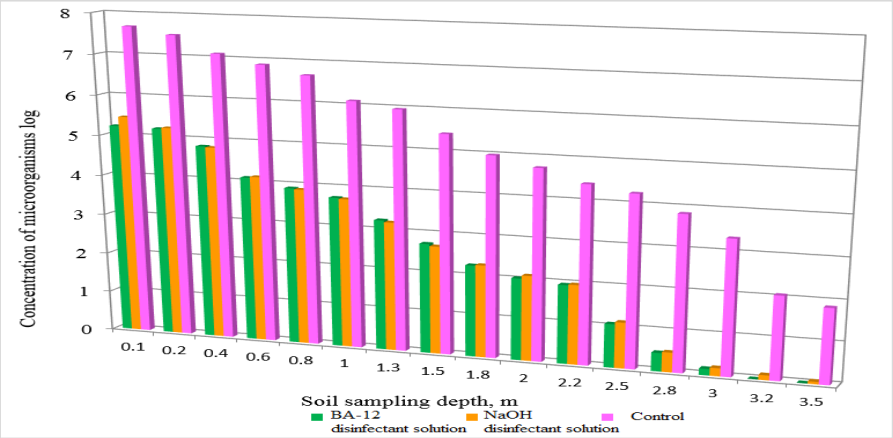

Fig. 3. Concentration of microorganisms, from the soil, after 12 hours of treatment with various disinfectants.

Thus, the BA-12 disinfectant has bactericidal and sporicidal properties and allows for the complete disinfection of anthrax burials to a depth of 3.5 m. This method does not require using expensive special equipment, the product is made in Kazakhstan, which is an important factor considering the geopolitical processes that may affect the supply of disinfectants from Russia to Kazakhstan, and this also reduces the cost of the preparation. The obtained

results of Ilyina *et al.* (2016) exhibit the nature of the effect provided by various doses of formaldehyde and toluene on the composition and functioning of the complex of soil microorganisms and display the mechanism of action of chemicals (formaldehyde and toluene) on the soil microbiota associated with its stability and the manifestation of soil toxicosis.

CONCLUSION

The conducted experiments allow us to draw the following conclusions.

1. The BA-12 disinfectant has bactericidal and sporicidal properties and allows for complete disinfection of anthrax burials to a depth of 3.5 m.

2. For complete and effective treatment to a depth of 3.5 m of soil foci with a gray-earth type of soil, the 50 cm distance between the pits is sufficient.

3. To disinfect a plot of 210.0 cm by 280.0 cm with a gray-earth type of soil, 12 wells by a distance of no more than 50 cm are needed. These criteria ensure horizontal dissociation of the solution and allow disinfecting the entire volume of the soil of the anthrax focus after double treatment.

4. Complete disinfection of the site with the above dimensions is achieved when it has been treated three times with an interval of 24 hours. The amount of BA-12 disinfectant solution is 1,635 L.

The results obtained will have practical conclusions since the developed scheme can be used in production work at existing anthrax burials that interfere with the prospective development of the region and require urgent liquidation.

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