VETERINARY PHARMACOLOGY

Рус. Eng.

UDC: 666.322.4: 615.281.9

DOI: 10.18413/2500-235X -2016-2-3-63-74

Bui Quang Cu [1], Nguen Hoai Chao[2], Vesentsev A.I.[3], Buhanov V.D. [4], Sokolovsky P.V.[5], Mihaylyukova M.O.[6]

THE ANTIBACTERIAL PROPERTIES OF MODIFIED BENTONITE DEPOSIT TAM BO

1) Doctor of technical sciences, professor, researcher, Institute of environmental technology-vietnam academy of science and technology. 18 Hoang quoc viet – Hanoi, Vietnam, e-mail: buiquangku@yahoo.com

2) Doctor of physico-mathematical sciences, professor, Head of Eco-friendly department, Institute of environmental technology-vietnam academy of science and technology. 18 Hoang quoc viet – Hanoi, Vietnam, e-mail: nhchau.iet@gmail.com.

3) Doctor of technical sciences, professor, head of the department of general chemistry, National Research Belgorod State University. 85, Pobedy St., Belgorod, 308015, Russia. e-mail: vesentsev@bsu.edu.ru.

4) Candidate of veterinary sciences, associate professor of medical and biological bases of physical education National Research Belgorod State University. 85, Pobedy St., Belgorod, 308015, Russia. e-mail: valabu55@bk.ru.

5) Graduate student, department of general chemistry, National Research Belgorod State University. 85, Pobedy St., Belgorod, 308015, Russia. e-mail: levap90@list.ru

6) Graduate student, department of general chemistry, National Research Belgorod State University. 85, Pobedy St., Belgorod, 308015, Russia. e-mail: 1158878@bsu.edu.ru

Abstract.
In this article, the antibacterial properties enriched bentonite Tam Bo deposit, Lam Dong Province (Vietnam) modified in various ways: alkali – by processing enriched bentonite LiOH solution at a temperature of 60°C for 30 min, brine – by processing enriched bentonite, 3% solution of Na_2CO_3 at 55°C for 30 min, and nanoparticles of silver – silver nanoparticles by immobilizing on the surface of the bentonite by precipitation of silver nanoparticles $AgNO_3$ solution. Initially, the bentonite clay deposits Tam Bo contains 40 – 60 wt% of active sorption-montmorillonite. Enrichment, followed by modifying the content of montmorillonite can increase up to 70 – 80 wt. %. The modification allows the bentonite increase in its physical-chemical and colloid-chemical characteristics to 2 – 3 times, such as degree of swelling, the specific surface area, cation exchange capacity. The experimental data showed that the bentonite modified with silver nanoparticles can effectively inhibit the growth of opportunistic pathogens like Enterococcus feacalis, Escherichia coli, Proteus mirabilis, Pseudomonas aerugenosa, Salmonella typhimurium, Staphylococcus aureus and Candida albicans. Modification of bentonite clay and montmorillonite crystal lattice is included in its composition with silver nanoparticles allows increasing the bactericidal activity against aflatoxin to 65 times.

Keywords: bentonite, montmorillonite containing sorbent, modification, enterosorbent, aflatoxin, pollutants, nano particle silver, opportunistic pathogens.

Introduction.

The main directions of modern biotechnology include the development of sorption materials for the purpose of their further use in the design of high-performance products for hemodialysis and enterosorption, preparations of immobilized enzymes, test kits for enzyme immunoassay and immunofluorescence analysis. Chelators are used to bind metabolites, toxins and other substances in the digestive tract are promising in addressing human food regulatory issues, to reduce the intake of environmentally harmful substances (including radionuclides, pesticides, heavy metals), prevention and treatment of diseases. Chelators are widely used in animal production [1-4]. Complex pathologic changes in the composition of the intestinal microflora with relevant clinical manifestations associated with dysbiosis, develops due to the use of antibiotics is leveled exclusively at the expense of application enterosorbents [5]. In this situation, a serious alternative to antibiotics are the methods of efferent medicine, allowing to correct the condition of the patient and reduce the toxic load on the body. Creating a comprehensive veterinary drugs, using different sorbents groups in combination with antibiotics, it extends the capabilities of extracorporeal methods of complex treatment of acute intestinal infections, as chelators are the means to a multi-faceted performance is determined not only by their symptomatic (antidiarrheal) and pathogenetic (detoxification, and others.) etiotropic and effect as against pathogenic bacteria and viruses.

They are used to increase productivity, and most importantly, reduce the cost of production due to the higher efficiency of the use of nutritional food substances. This transformation is achieved by increasing the feed of nutrients in the products due to the use of advanced technology for feeding feed preparations and additives stimulating the digestibility and utilization of nutrients. One of the promising mineral adsorbents, are used as feed additives are natural clays containing montmorillonite [6-8]. The main purpose of them – animals detoxification of the body is achieved by enterosorption pathogens organic and inorganic nature. This feed supplement contains no chemicals that negatively affect the quality of animals and products derived from them. It is not toxic and has no cumulative properties. Natural minerals containing rich composition of micro- and macronutrients, help to improve digestion and utilization processes of nutrients, thereby increasing resistance and productivity of animals. The study shows that the animals are removed from the body of the missing minerals chemical elements, giving through the ion exchange mechanism redundant, thereby normalizing the mineral balance.

For example, a feed additive «M-Feed» (France manufacturer, Za du Haut du Bois 56580 Brehan France) on the basis of montmorillonite clay is designed to improve the quality of feed and improve the work of the gastrointestinal tract of the animal. This supplement is a combination high-tech and totally natural product, created using nanotechnology. Penetrating into the animal together with fodder, it absorbs harmful substances in the gastrointestinal tract; adsorbs large molecules secreted by pathogenic bacteria, mycotoxins and heavy metals; It stimulates specific and non-specific local immunity of the animal, and normalizes the viscosity of the intestinal contents, protects the intestinal walls and helps to normalize the intestinal microflora. Extracts of herbs and essential oils that make up the «M-Feed», causes appetite, improves the palatability of feed, promote better its digestibility and protect the body against pathogenic bacteria and parasites.

Numerous studies have confirmed that the addition of montmorillonite clay in animal feed increases milk yield and milk fat content, quality and taste of meat in cattle; It provides an increase in weight – in pigs; egg production and egg quality – a bird [9-12], improves some biochemical parameters of blood, in particular, increases the content of calcium, magnesium, inorganic phosphorus [13].

In the digestive tract the bentonite absorbs water and digestive juices, this increases the surface exposure of food bacteria, which improves the digestibility of food. Moreover, thanks to the ability of the bentonite to the selective adsorption of the chemical elements are removed from the digestive system of toxic substances, bacteria, alkaloids protein compounds) [14].

Prevalence containing montmorillonite clay is quite high. There are a number of large deposits containing montmorillonite clay around the world [15-19]. An urgent task of research is to develop methods for the modification of montmorillonite clay to improve their sorption characteristics and bactericidal properties. Finding the solution of this problem is devoted to this article. As a material for the study was taken bentonite clay deposits Tam Bo province of Lam Dong (Vietnam). Currently, the deposit Tam Bo bentonite clay is mined from the quarry area 5,9 km^2. Crude bentonite imeeet heterogeneous and different shades from light gray to yellowish. According to the Vietnamese geologists bentonite reserves in the deposit, Tam Bo is 178 million m^3, including 59 million m^3 montmorillonite.

Material and methods of research.

For the study in this paper are taken samples of bentonite clays Tam Bo deposit, which is assigned to the following markings TN 1, TN 5/1, VT 6. All samples were fine powders grayish-yellow color. The mineralogical and chemical composition of bentonite clay Tam Bo deposit was determined by X-ray and X-ray fluorescence analysis using X-ray workstation ARL 9900 series x-ray workstation with Co anode. Morphological characteristics of crystals included in the study clays determined using analytical transmission electron microscope of high resolution JEOL-2100 (Japan), and a deposit emission transmission electron microscope ultrahigh resolution Technai G2F20 S-Twin, FEI Company (Netherlands). To investigate the montmorillonite clay used a special method of sample preparation for non-self-supporting samples and suspensions [20]. The carbon used as the substrate film which has been obtained by spraying a spectrally pure carbon layer on the surface of the single crystal halite. Directly before spraying halite crystal cleaved along cleavage planes for obtaining clean smooth surface. Spraying was carried out with the device for the deposition of carbon films in vacuum Quarum Q150RE. Pulse sputtering was performed in a vacuum (pressure not exceeding 10.04 mbar) did 2-4 pulse for 2-5 seconds with 30 seconds intervals. Cooked lowered carbon film on the surface of distilled water, poured into a short flat cylinder with a large free surface area.

Clutching a copper mesh with tweezers, gently caught carbon film so that it covered the mesh. Further, the mesh was placed in a box for storage meshs. To the resulting mesh with a carbon film deposited drop of an aqueous suspension of the sample. Moreover, according to the guidelines of [21] to obtain reliable results of the study, analysis of the samples was performed for three reticula sample deposited from the same sample, after thorough mixing, each sample prior to application to the mesh. According to the guidelines of the [22] The laboratory sample was formed in the amount of allocation of the average sample sufficient to perform a single analysis.

Enrichment of bentonite was carried out by precipitation from an aqueous slurry, pouring the slurry of the upper layer, followed by drying. Further carried out the modification of bentonite clay. For convenience, the notation method of modifying clays samples: alkaline – Li, salt – Na, the modification of silver nanoparticles – Ag. Enrichment clay identified – En (sample name), the native form of clay identified – Nat (sample name).

The modification was carried out in the following ways:

- Alkali (Li): bentonite enriched by treatment with LiOH solution at 60°C for 30 min.

- Salt (Na): by treating bentonite enriched with 3% solution of Na_2CO_3 at 55°C for 30 min.

- Silver nanoparticles (Ag): by immobilizing the silver nanoparticles of bentonite surface by deposition of silver nanoparticles $AgNO_3$ solution. Application of Silver Nanoparticles to bentonite is as follows: bentonite clay in powder form is suspended in an aqueous solution of silver nitrate, and the Ag^+ ions are uniformly distributed in the pores of montmorillonite, including inter-packet space lattice montmorillonite as laminate silicate structure type 2: 1 crystal lattice swelling. Then, the silver ions were reduced to nano-sized metallic state using $NaBH_4$.

Adsorption characteristics of bentonite clay samples are determined in relation to the heavy metal ions (Pb^{2+} and Cd^{2+}) by spectrophotometry.

Sensitivity Enterococcus feacalis, Escherichia coli, Proteus mirabilis, Pseudomonas aerugenosa, Salmonella typhimurium, Staphylococcus Aureus and Candida albicans to the experimental samples performed sorbents meat infusion agar twice dilution method (MIA) containing 2 wt. % Agar. Clays investigated sample was placed into tubes and sterilized in an oven at a temperature of 160-180°C. Then these tubes were added to 5 ml of molten MIA. In the molten MIA, the temperature of which was 42 – 43°C, the slurry was poured in advance of one of the strains of microorganisms, the rate of $1*10^7$ CFU (colony forming units) in 1 ml of MIA.

In a control tube was added only to the MIA study the culture of microorganisms, and in the next – only the MIA with the test Hinge of clay.

The contents were then carefully resuspended and tubes were placed in a special holder to obtain MIA beveled surface. After sealing agar experimental and control tubes were placed in an incubator, and cultured at 37°C for 16-18 hours.

After culturing with the slant surface, followed by washings were prepared in determining concentration of microorganisms through the optical device – «Densilametr». Performance of the device in the control swabs tubes, which contained MIA and studied clay, used to adjust the true concentration of microorganisms in the control tubes swabs, because Optical turbidity difference, expressed in units of McFarland in washouts experimental and control tubes reflect the true concentration of microorganisms in 1 ml. Washes produce sterile isotonic sodium chloride solution, the volume of which was 5 ml.

Study of the sorption capacity of bentonite clays in relation to mycotoxins performed as follows: a sample of 10 mg of study 0,2 bentonite clay was placed in a beaker containing 10 ml of a solution of aflatoxin B1 (AfB1) with a concentration of 59 mg/l, pH = 7.0. After stirring, shaking for 60 min, the solution was left for 24 hours for precipitation. The clarified solution was separated and the upper part was collected to determine the content of it remaining AfB_1 ELISA method using a test-kit AgraQuant Aflatoxin B_1 firm Romer Labs (USA). To carry out the experimental work bution effectiveness inhibition of development of mycotoxins using bentonite clay modified with silver nanoparticles following experimental parameters were used as a producer of the mycotoxin using mold biomass peanuts on a culture medium Sabouraud (4% agar from glukoza) from Merck (Germany) . bentonite nanosilver concentration was 2 wt.%. The content of nanosilver in the bentonite clay in the trials was 0; 5; 10; 20; 30 and 50 mg in 10 ml culture medium Sabouraud (4% glucose agar). Fungal spores were transferred into Petri dishes with Sabouraud culture medium (4% glucose agar) containing bentonite clay modified with silver nanoparticles. The plates were placed in a thermostated oven for 24 – 48 h at 35°C. Circle diameter determined spread of fungi in a petri dish to determine the efficacy of inhibition of fungi under the influence of bentonite clay modified with silver nanoparticles.

Results and discussion.

The first stage of implementation of the work was to study the material composition of the clay samples submitted. Oxide chemical composition shown in table 1.

Table 1

Chemical composition of the oxide samples of clay from Tam Bo deposit

Sample	The content of oxides by wt.%														
	SiO_2	Al_2O_3	Fe_2O_3	MgO	CaO	TiO_2	K_2O	MnO	Na_2O	Rh_2O_3	V_2O_5	Cr_2O_3	ZrO_2	ZnO	п.п.п.	Sum
TN 1	51,72	21,49	9,46	2,65	1,83	1,43	0,70	0,075	0,063	0,047	0,028	0,023	0,021	0,01	10,45	100
St.error, %	0,18	0,14	0,12	0,12	0,024	0,07	0,0019	0,044	0,0038	0,0015	0,0008	-	0,018	-	0,02	-
TN 5/1	55,30	22,31	6,48	2,76	0,74	0,67	1,36	0,018	0,049	0,028	0,016	-	0,016	-	10,25	100
St.error, %	0,18	0,14	0,12	0,12	0,024	0,07	0,0019	0,044	0,0038	0,0015	0,0008	-	0,018	-	0,02	-
VT 6	56,62	20,90	6,71	2,26	0,62	0,75	1,38	0,040	0,067	0,016	0,019	-	0,017	0,011	10,58	100
St.error , %	0,24	0,21	0,13	0,08	0,034	0,042	0,06	0,002	0,005	0,002	0,021	-	0,001	0,001	0,05	-

Submitted samples contain in its structure silicas, aluminum and calcium (SiO_2, Al_2O_3 and CaO), are typical for bentonite clay-based minerals montmorillonite groups, namely, dioctahedral montmorillonite aluminum ions, alkaline earth metal ions, in this case Ca^{2+}. Fairly high content of aluminum oxide is typical for montmorillonite, kaolinite clays. The test samples of bentonite clay content of iron oxide (6.71 – 9.46 wt.%), slightly more than the previously published data [23, 24]. The mineralogical composition of the investigated clays are presented in Table 2.

Table 2

Mineralogical composition clay samples

Minerals	Content, wt.%		
	TN 1	TN 5/1	VT 6
Montmorillonite	51	55	45
Kaolinite	11	12	12
Illite	9	10	10
Dolomite	4	1	4
Quartz	10	10	21
Muscovite	5	3	missing
Feldspar	trace	trace	trace
Goethite	3	5	3
Chlorite	4	4	3
Sum	100	100	100

The mineralogical composition clay samples from Tam Bo deposits submitted to montmorillonite (45-55 wt.%), Kaolinite (11-12 wt.%), Illite (9-10 wt.%), Low temperature trigonal quartz (10-21% by weight.) goethite, feldspars, chlorite, and others. It is found that the content of montmorillonite – essential mineral determining the adsorption capacity of bentonite clay – varies depending on the sampling site. The content of montmorillonite clay deposit samples Tam Bo, selected near the reservoir, up to 70 wt.%. However, the average content of montmorillonite clays from Tam Bo deposit is within 50 wt.%. Based on these data we can conclude that bentonite from Tam Bo deposit can be used for sorption-active material with high sorption capacity, suitable for the preparation of feed additives used in animal husbandry and particularly in poultry [9-12].

The study of structural and morphological characteristics of the deposits of bentonite clays samples from Tam Bo deposit revealed that the distribution of crystal montmorillonite and related minerals in clays uneven (Fig. 1 a). An alternation of individual crystals montmorillonite (S) and its components (A). The size of the aggregates is 10-70 microns (Figure 1 b).

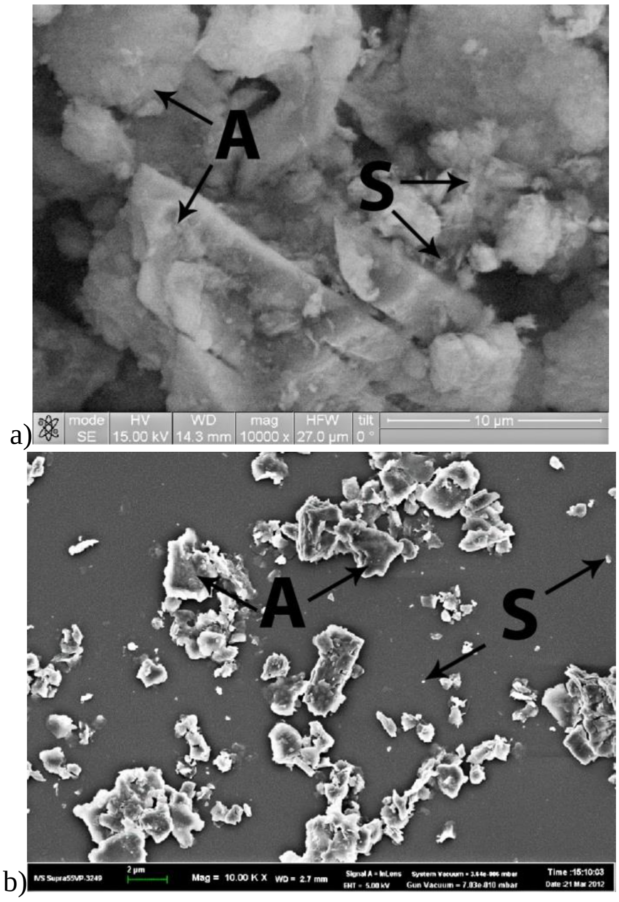


Figure 1. The distribution of particles in a sample of montmorillonite clay VT 6 from Tam Bo deposit (S – montmorillonite individual crystals; A – montmorillonite aggregates of crystals)

It can be seen (Figure 2.), that the form of montmorillonite crystals are plate isometric shapes, sizes 1-4 microns.

On the surface of montmorillonite crystals crack unstable fixed mold (F), a thickness of 5 nm and a length of 40 to 50 nm (Fig. 2a). Fixed to the inner surface cracks (Fig. 2 a) may be introduced and various sorbed pollutants having a size less than 5 nm. montmorillonite crystals are torn, jagged edges (Fig. 2 b). The edges of the crystals twist in tubes (T).

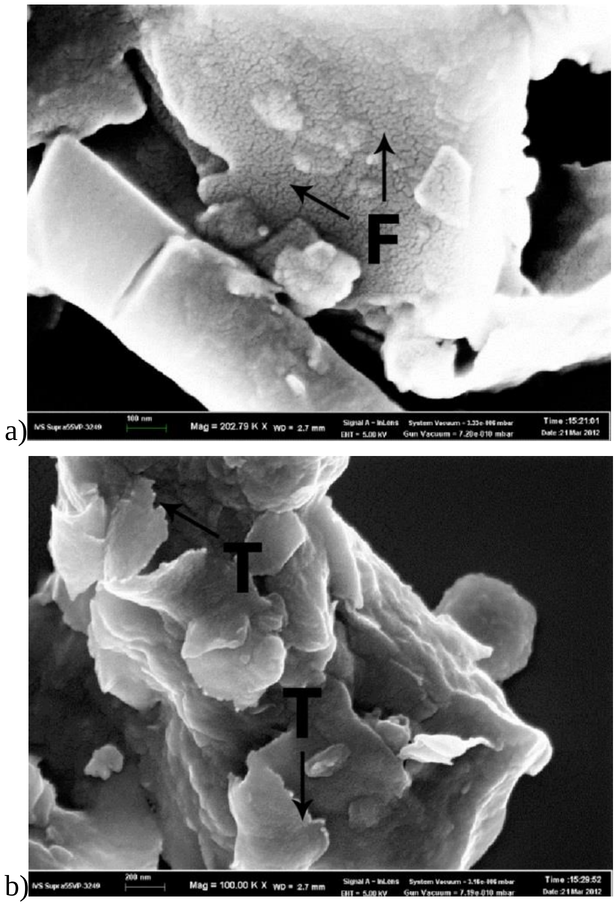


Figure 2. deposits crystals in a sample of montmorillonite clay VT 6 from Tam Bo deposit (F – crack unstable forms, T – twisted into a tubes edge crystal montmorillonite)

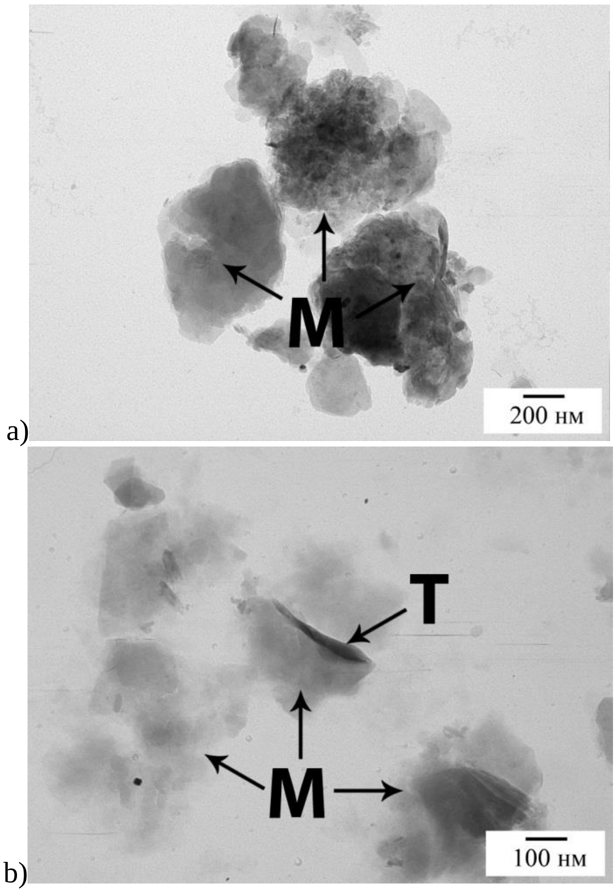


Figure 3. Montmorillonite (M) of sample clay VT 6 from Tam Bo deposit, T- twisted into a tubes edge of montmorillonite crystal

Morphological characteristics of individual particles of fine clay and related minerals determined by transmission (transmission) electron microscopy (Figure 3-6). It is found that crystal particles are montmorillonite isometric views of films of size 100-200 nm, which tend to assemble into aggregates. The thickness of the crystals determined by montmorillonite from darkdeposit transmission electron microscopy studies and of 3-5 nm.

The methods of transmission electron microscopy confirmed that the edge nanoplenochnogo montmorillonite single crystal can twist (Fig. 3), which is probably due to the energetically more favorable state, because of the surface tension forces are balanced. montmorillonite crystals can twist in tubes, which is probably due to a thermodynamically stable state of a cylindrical shape compared with the film (Fig. 3). This process intensifies the action of the flow of electrons.

Low temperature trigonal crystal is shown in Figure 4 as a massive dark crystals.

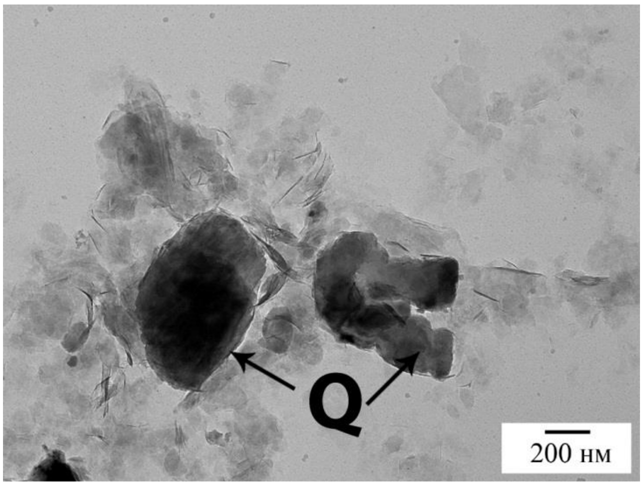


Figure 4. Electron micrograph of a low-temperature trigonal quartz (Q) from a sample of clay VT 6 from Tam Bo deposit

Also kaolinite crystals are present in the composition of clay, having the form of hexagonal plates and pretty clear crystallographic facet (Figure 5).

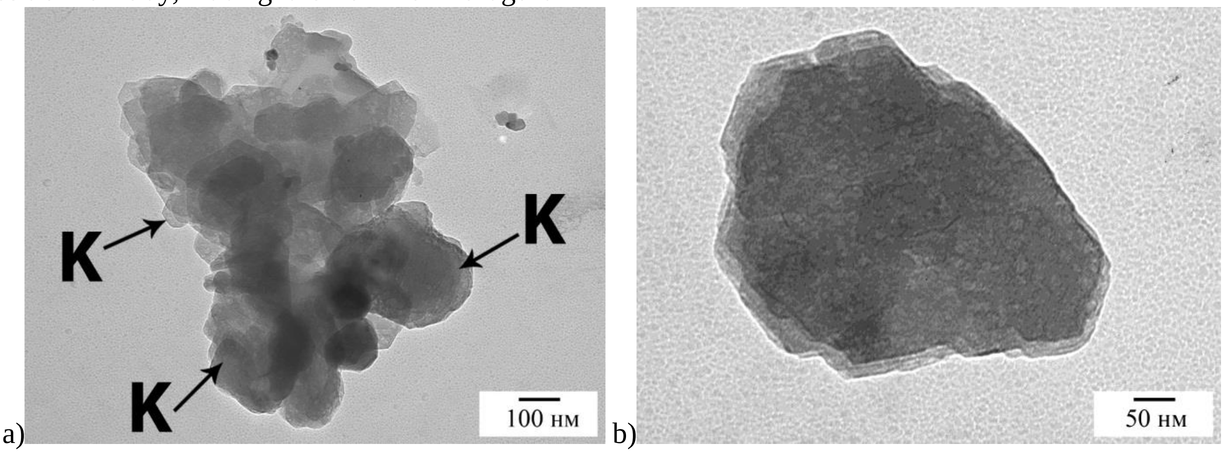


Figure 5. Kaolin (K) from the sample of clay VT 6 from Tam Bo deposit.

Figure 6 shows the results of experimental studies on the adsorption Pb^{2+} ions and Cd^{2+} in the original sample bentonite VT 6 (Fig. 6 a) and enriched (Fig. 6 b), depending on the exposure time. It is found that the adsorption begins from the first moment of interaction, and reaches a maximum after 30 minutes. The adsorption capacity of Pb^{2+} and Cd^{2+} in samples of bentonite clay depends on pH and increases with increasing pH from 2 to 6. In the region of pH 6 these metals undergo hydrolysis and form precipitates, whereby the adsorption process is terminated.

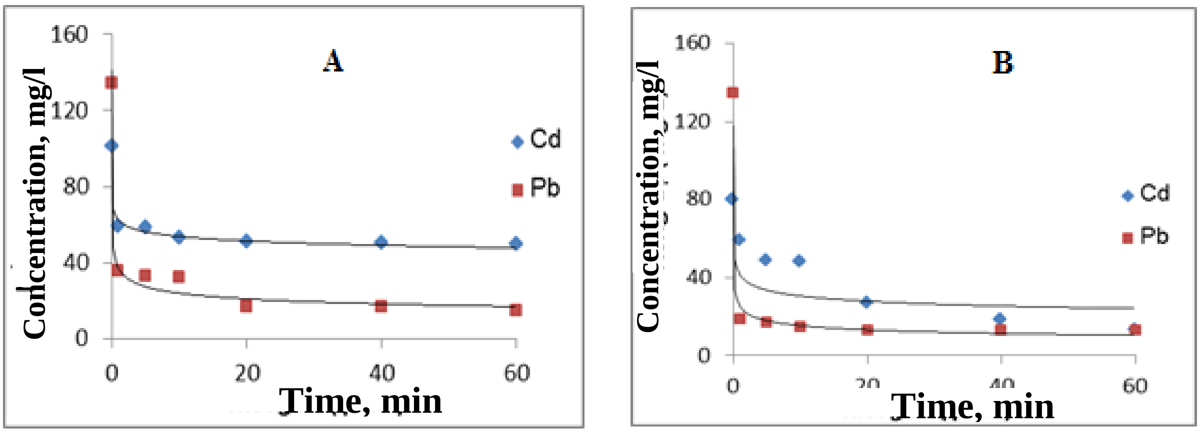


Figure 6. Dependence of concentration changes of the ions Pb^{2+} and Cd^{2+} on the adsorption on the duration of the original bentonite clay samples VT6 (A) and rich bentonite clay samples VT6 (B)

Table 3 presents the results of a study of sorption activity with respect to the ions Pb^{2+} and Cd^{2+} depending on the method of modification of bentonite clay.

Table 3

Adsorption of ions Pb^{2+} and Cd^{2+} on experimental samples

Samples	Adsorption capacity, mg/g	
	Pb^{2+}	Cd^{2+}
Nat (VT 6)	24,6	11,2
En (VT 6)	63,9	26,6
Na (VT 6)	62,7	29,5
Ag (VT 6)	58,8	26,0

From Table 3 it is established that the modification of bentonite clays in various ways enhances its adsorptive capacity of clay with respect to ions Pb^{2+} and Cd^{2+} from 2 – 3 times.

Table 4 shows the results of determination of the sorptive capacity of clays from Lam Dong Province towards pathogenic microorganisms.

Table 4

The sorption capacity Tam Bo clay deposits in relation to conditionally pathogenic microorganisms

Microorganisms	Clay concentration in mg/ml in MIA	Native clay samples			
		TN 1	TN 5/1	VT 6	Control
		Cfu / ml			
Enterococcusfeacalis	400	$9 \cdot 10^{8}$	$1 \cdot 10^{8}$	$1 \cdot 10^{8}$	$2 \cdot 10^{8}$
	200	$7 \cdot 10^{8}$	$2 \cdot 10^{8}$	$2 \cdot 10^{8}$	
	100	$9 \cdot 10^{8}$	$2 \cdot 10^{8}$	$3 \cdot 10^{8}$	
Escherichia coli	400	$12 \cdot 10^{8}$	$2 \cdot 10^{8}$	0,0	$3 \cdot 10^{8}$
	200	$13 \cdot 10^{8}$	$2 \cdot 10^{8}$	0,0	
	100	$21 \cdot 10^{8}$	$3 \cdot 10^{8}$	$2 \cdot 10^{8}$	
Proteus mirabilis	400	$2 \cdot 10^{8}$	$1 \cdot 10^{8}$	0,0	$5 \cdot 10^{8}$
	200	$3 \cdot 10^{8}$	$1 \cdot 10^{8}$	$2 \cdot 10^{8}$	
	100	$5 \cdot 10^{8}$	$2 \cdot 10^{8}$	$10 \cdot 10^{8}$	
Pseudomonas aerugenosa	400	$3 \cdot 10^{8}$	$1 \cdot 10^{8}$	$2 \cdot 10^{8}$	$5 \cdot 10^{8}$
	200	$3 \cdot 10^{8}$	$1 \cdot 10^{8}$	$3 \cdot 10^{8}$	
	100	$5 \cdot 10^{8}$	$3 \cdot 10^{8}$	$3 \cdot 10^{8}$	
Salmonella typhimurium	400	$6 \cdot 10^{8}$	$1 \cdot 10^{8}$	$3 \cdot 10^{8}$	$4 \cdot 10^{8}$
	200	$8 \cdot 10^{8}$	$2 \cdot 10^{8}$	$4 \cdot 10^{8}$	
	100	$9 \cdot 10^{8}$	$3 \cdot 10^{8}$	$5 \cdot 10^{8}$	
Staphylococcus aureus	400	$6 \cdot 10^{8}$	0,0	0,0	$3{,}5 \cdot 10^{8}$
	200	$9 \cdot 10^{8}$	$1 \cdot 10^{8}$	0,0	
	100	$9 \cdot 10^{8}$	$3 \cdot 10^{8}$	$1 \cdot 10^{8}$	
Candida albicans	400	$1 \cdot 10^{8}$	$1 \cdot 10^{8}$	0	$5 \cdot 10^{8}$
	200	$3 \cdot 10^{8}$	$5 \cdot 10^{8}$	$1 \cdot 10^{8}$	
	100	$3 \cdot 10^{8}$	$6 \cdot 10^{8}$	$3 \cdot 10^{8}$	

Following the information given in the table, it should be concluded that seven Vietnamese containing montmorillonite clay the most effective bacteriostatic effect in respect of the studied bacteria and microscopic fungi of the genus Candida, had clay samples TN 5/1 and VT 6. In this test TN5/1 suppressed the growth of Staphylococcus aureus (clay concentration of 400 mg/ml MIA), and showed clay VT 6 effective inhibitory effect on Escherichia coli (400 mg/ml MIA), Proteus mirabilis (400 mg/ml MIA), Staphylococcus aureus (200-400 mg/ml MIA) and Candida albicans (200-400 mg/ml MIA).

Less productive remaining samples were clays which are not delayed growth on investigated microorganism strains MIA containing a high concentration (400 mg / ml) of test samples (TN 1) and stimulate the growth and reproduction of microorganisms.

For example, the number of microorganisms in the test tubes with clay TN 1 greater than the number of Cfu of microorganisms grown in test tubes in 1.5 – 7 times. The stimulation of microbial growth is probably due to the presence of clay minerals, contributing to their growth.

As the object of study to take a sample of bentonite clay VT 6, which showed the highest antibacterial properties. To enhance the antibacterial activity conducted most effectively modifying clay sample VT 6 different ways: saline (Sample Na (VT 6)), alkaline (Sample Li (VT 6)) and the silver nanoparticles (Sample Ag (VT 6)). As a control, the activated carbon is taken pharmaceutical, manufacturing Pharmstandard, Russia. antibacterial activity results are shown in Table 5.

Table 5

Relationship of microorganisms washings concentration (Cfu / ml) on the presence of the ingredients in the composition and quantitative content of sorbent

Microorganisms	Clay concentration in mg/ml in MIA	Modificated clay samples			Control
		Na (VT 6)	Li (VT 6)	Ag (VT 6)	
Escherichia coli	200	$18 \cdot 10^8$	0	0	$39 \cdot 10^8$
	100	-	0	0	
	50	-	0	0	
	25	-	*	0	
	12,5	-	$24 \cdot 10^8$	*	
	6,25	-	-	$2 \cdot 10^8$	
	3,125	-	-	$18 \cdot 10^8$	
Salmonella dublin	200	$15 \cdot 10^8$	0	0	$12 \cdot 10^8$
	100	-	0	0	
	50	-	*	0	
	25	-	$9 \cdot 10^8$	0	
	12,5	-	-	0	
	6,25	-	-	*	
	3,125	-	-	$2 \cdot 10^8$	
	1,56	-	-	$9 \cdot 10^8$	
Salmonella enteritidis	100	$18 \cdot 10^8$	0	0	$22 \cdot 10^8$
	50	-	0	0	
	25	-	*	0	
	12,5	-	$6 \cdot 10^8$	0	
	6,25	-	-	*	
	3,125	-	-	$2 \cdot 10^8$	
	1,56	-	-	$18 \cdot 10^8$	
Staphylococcus hyicus	200	$30 \cdot 10^8$	0	0	$36 \cdot 10^8$
	100	-	0	0	
	50	-	0	0	
	25	-	$2 \cdot 10^8$	0	
	12,5	-	$30 \cdot 10^8$	0	
	6,25	-	-	*	
	3,125	-	-	$6 \cdot 10^8$	
Staphylococcus intermedius	200	$18 \cdot 10^8$	0	0	$30 \cdot 10^8$
	100	$36 \cdot 10^8$	0	0	
	50	-	$3 \cdot 10^8$	0	
	25	-	$21 \cdot 10^8$	0	
	12,5	-	-	*	
	6,25	-	-	$2 \cdot 10^8$	
	3,125	-	-	$24 \cdot 10^8$	
Staphylococcus aureus, метициллин резистентный	200	$33 \cdot 10^8$	0	0	$36 \cdot 10^8$
	100	-	0	0	
	50	-	0	0	
	25	-	$2 \cdot 10^8$	0	
	12,5	-	$20 \cdot 10^8$	*	
	6,25	-	$22 \cdot 10^8$	$2 \cdot 10^8$	
	3,125	-	-	$21 \cdot 10^8$	
Proteus vulgaris	12,5	-	-	-	$36 \cdot 10^8$
	6,25	-	-	*	
	3,125	-	-	$2 \cdot 10^8$	
	1,56	-	-	$18 \cdot 10^8$	

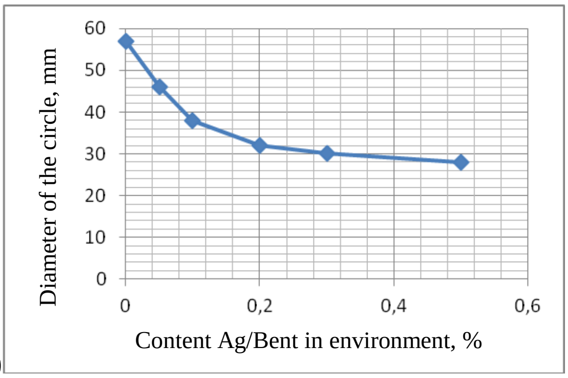
Note: 0 – the presence of microbial growth; * – Bactericidal effect; – - The study was conducted.

It is found that the sample Li (VT 6) at a dose of 25 and 50 mg/ml bactericidal acted on Salmonella and Escherichia. In this form of staphylococcus sorbent at concentrations of 50-100 mg/ml provided only bacteriostatic effect, as after the sowing of the resulting washings are always marked growth of staphylococci used.

It is necessary to note the fact that the sample of Na (VT 6) at a content of 200 mg/ml MIA not inhibited growth of test organisms. In turn, it enhances the growth of Staphylococcus intermedius and Salmonella dublin. Compared to control the number of microbial cells per 1 ml wash it was more respectively 1.2 and 1.3 times. Also increased the number of Cfu sorbent Staphylococcus aureus in 1 ml wash 1.1 times.

More effectively inhibit the growth of bacteria in the study and act on bacteria bactericidal effect sample Ag (VT 6) in concentrations 6.25 – 12.5 mg/ml MIA. The control sample at a concentration of 100 mg/ml MIA did not suppress the growth and development of microbial strains investigated (tabl. 4). Instead inhibition of their growth and development, he on the contrary stimulate their proliferation. Guided by the results obtained, it should be noted the high efficiency of the sorbent shaped silver Ag (VT 6). Designed complex preparation can be widely used in agriculture and veterinary.

The study of the ability of modified clay to inhibit the growth of fungi and the effect of mycotoxins. Figure 7 shows the results of determining the efficiency of inhibition of fungi under the influence of bentonite clay, modified nanoparticulate silver (Ag (VT 6)). The weight ratio of the modified silver / Bentonite clay nanoparticles (Ag / Bent) / culture medium was varied between 0.05-0.5 wt.%.

a) 

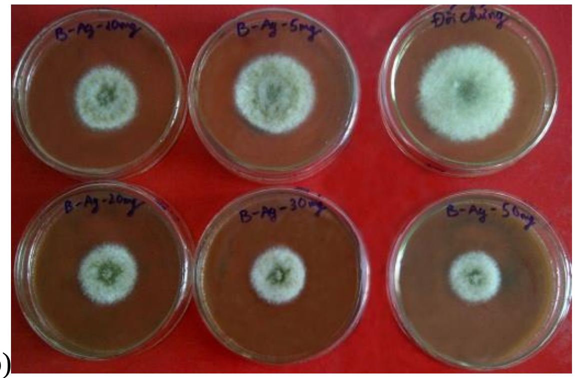
b) 

Figure 7. Effect of fungal growth inhibition sorbent Ag (VT 6) depending on the weight ratio of Ag / Bent

It was found that immobilized nanosilver bentonite clay are well inhibits the growth of fungal mycotoxins. In Figure 7 it can be seen that increasing the ratio of Ag / Bent from zero to 02 wt. % Circle diameter of fungal growth is significantly reduced, indicating that the inhibitory effects of silver nanoparticles with respect to mycotoxins.

The adsorption of aflatoxin B1 (AfB_1) samples of bentonite clays modified with other methods conducted in water (bentonite clay concentration of 0.1 wt.%). AfB_1 residual concentration in the filtrate was analyzed by ELISA. The results are shown in Table 6.

Table 6

Efficacy AfB1 adsorption on a sample of bentonite clay in water at AfB_1, initial concentration 59 mg/l

Samples	Concentration AfB_1 (mg/l)	Adsorption efficiency (%)
Nat (VT 6)	59,00	0
En (VT 6)	6,590	88,8
Na (VT 6)	0,268	99,5
Li (VT 6)	0,105	99,8
Ag (VT 6)	0	100

It was established that the modification of bentonite clay VT 6mestorozhdeniya Tam Bo leads to a substantial increase in the ability to inhibit the growth of fungi and aflatoxin absorption (up to 65 times).

CONCLUSION

Established material (chemical, mineralogical) composition, as well as the structural and morphological characteristics of the deposits of clay samples Tam Bo Lam Dong province. The mineralogical composition of the clay samples (TN 1, TN 5/1, VT 6) Tam Bo deposits represented montmorillonite (45 – 55 wt.%), Kaolinite (11 – 12 wt.%), Illite (9 – 10 wt.%), trigonal quartz low temperature (10 – 21 wt.%), goethite, feldspars, chlorite, and others. It is found that the content of montmorillonite – essential mineral determining the adsorption capacity of bentonite clay – varies depending on the sampling site. The content of montmorillonite clay deposit samples Tam Bo, selected near the reservoir, up to 70 wt.%. However, the average content of montmorillonite clays Tam Bo deposit is within 50 wt.%. A modification of the clay samples presented in different ways: alkali – bentonite enriched by treatment with LiOH solution at 60°C for 30 min, brine – bentonite enriched by treatment with 3% solution of Na2CO3 at 55°C for 30 minutes and the silver nanoparticles – by immobilization of silver nanoparticles on the surface of the bentonite by the deposition of silver nanoparticles of $AgNO_3$ solution. It was found that at a dose of 25 and 50 mg/ml of the alkaline form of clay (sample Li (VT 6)) bactericidal effect on Salmonella and Escherichia. In this form of staphylococcus sorbent at concentrations of 50-100 mg/ml provided only bacteriostatic effect, as after the sowing of the resulting washings are always marked growth of staphylococci used.

Clay treated with saline method (sample Na (VT 6)) at a content of 200 mg/ml MIA, not inhibit the growth of bacteria in the study. In turn, it enhances the growth of Staphylococcus intermedius and Salmonella dublin. Compared to control the number of microbial cells per 1 ml wash it was more respectively 1.2 and 1.3 times. It also increases the number of Cfu of Staphylococcus aureus in 1 ml wash 1.1 times. The most effective proved to be the clay modified with silver nanoparticles (sample Ag (VT 6)). Even at concentrations 6.25 – 12.5 mg/ml MIA, it inhibits the growth of opportunistic microorganisms. The control sample at a concentration of 100 mg/ml MIA did not suppress the growth and development of microbial strains investigated (tab. 4). Instead inhibition of their growth and development, he on the contrary stimulate their proliferation.

It was established that the modification of bentonite clay VT 6mestorozhdeniya Tam Bo leads to a substantial increase in the ability to inhibit the growth of fungi and aflatoxin absorption (up to 65 times). The most effective in inhibiting the growth of fungi sorbent is modified with silver nanoparticles.

FINDINGS

The experimental results showed that the modified clay Tam Bo deposit, Lam Dong province (Vietnam): En (VT 6), Na (VT 6), Li (VT 6), Ag (VT 6) can be used as enterosorbent for detoxification humans and animals from ions of heavy metals, pathogenic and opportunistic bacteria, mycotoxins, and hence in the production of feed additives for livestock and ptitsevodstvodcheskih.

Work is performed at the expense of means of a grant of the Russian Federal Property Fund № 14-43-08021 «Research of processes phaseformation and structurizations proceeding at joint pyrolysis of vegetable waste of agro-industrial complex of the Belgorod region with local montmorillonite the containing clays and studying of influence of physical and chemical parameters of process of synthesis of effective composite sorbents on absorption of heavy metals, pathogenic and opportunistic bacteria from water solutions and cleaning of fertile soils of pesticides», 2015 – 2016, and State contract № 14.N08.11.0109 «Preclinical research of medical product – bactericide enterosorbent based on montmorillonite», 2016-2018, and region grant «Development of an complex medical veterinary product for treatment and prevention gastrointestinal infectious disease of pigs, produced from in expensive raw materials of Belgorod region», 2016-2017.

ЛИТЕРАТУРА

1. Abbès S., Z. Ouanes J. B. Salah-Abbes M. A. Abdel-Wahhab R. Oueslati H. Bacha. Preventive roles of aluminosilicate clay against induction of micronuclei and chromosome aberrations in bone-marrow cells of Balbc mice treated with Zearalenone. *Mutat. Res.* 631 (2007): 85-92 [PubMed] [Full text].

2. Abbès S., Z. Ouanes, J. B. Salah-Abbès, Z. Houas R. Oueslati, H. Bacha, O. Othman. The protective effect of hydrated sodium calcium aluminosilicate against haematological, biochemical and pathological changes induced by Zearalenone in mice. *Toxicon.* 47 (2006): 567-574 [PubMed] [Full text].

3. Farnworth E. R., H. L. Trenholm. The effect of acute administration of the mycotoxin zearalenone to female pigs. *J. Environ. Sci. Health B*, 16 (1981): 239-252 [PubMed] [Abstract].

4. Feng J., M. Shan, H. Du, X. Han, Z. Xu. In vitro adsorption of zearalenone by cetyltrimethyl ammonium bromide-natural montmorillonite nanocomposites. *Micropor. Mesopor. Mat.* 113(2008): 99-105 [Livestock Research for Rural Development] [Full text].

5. Wistrom J., Norrby S.R., Myhre E.B., Eriksson S., Granstrom G., Lagergren L., Englund G., Nord C.E., Svenungsson B. Frequency of antibiotic–associated diarrhea in 2462 antibiotic–treated hospitalized patients: a prospective study. *Journal of Antimicrobial Chemotherapy*. 47 (2001): 43-50 [PubMed] [Abstract].

6. M.Trckova, L. Matlova, L.Dvorska, I.Pavlik. Kaolin, bentonite, and zeolites as feed supplements for

animals: health advantages and risks. *Vet.Med. Czech* 49, 10 (2004): 389 – 399 [Books. Google][Abstract]

7. J. H. Quisenberry. The use of clay in poultry feed. *Clays and Clay Minerals*. 16 (1968): 267-270. [Clays and Clay Minerals][Full text]

8. Robert, A. Smectite-type clay minerals as nanomaterials, Schoonheydt. *Clays and Clay Minerals.* 50, 4 (2007): 411-420 [Reasearchgate][Full text].

9. V. D. Bukhanov, A. I. Vezentsev, O. N. Pankova, L. I. Naumenko, P. V. Sokolovsky. Influence montmorillonite of the containing clay on an organism of laying hens when receiving organic eggs. *Russian veterinary magazine. Series farm animals.* 4 (2015): 8-11 (In Russian) [Cyberlinka][Full text] .

10. V.D. Bukhanov, A.I. Vezentsev, A. A.Antipov, M. Z.Fyodorova, N. A. Volovicheva, N. F. Ponomaryova, N. A. Safonova, L. A. Kozubova. Use of the activated montmorillonitovy clay in a sharp experiment on the broilers infected kolibakteriozy and salmonellosis *Topical issues of veterinary biolog*y. 4, 12 (2011): 51-57 (In Russian) [Twirpx][Full text].

11. G.G. Onishchenko. Chemical safety as an interdepartmental problem. The role of the State Sanitary and Epidemiological Russia in chemical safety population. *Poison Gazette*, № 1 (2002): 2-7 (In Russian). [eLIBRARY][Full text]

12. Sycheva, L.V.. Stern and feeding of an agricultural bird: Monograph. Perm, Perm SAA (2010): 126 p. (In Russian) [RSL][Abstract].

13. Davydova S.L., Tagasov V.I. Heavy metals as supertoxicants XXI century: a training manual. *People's Friendship University* (2002): 140 (In Russian) [twirpx][Full text].

14. Rolfe B. N., Miller R. F., Mcqueen I. S. Dispersion characteristics of montmorillonite, kaolinite and hike clays in waters of varying quality, and their control with phosphate dispersants. *Geological Survey Professional Paper* 334-G (1960): 48 [Usgs][Full text].

15. Wai K.N, Banker G.S. Some physicochemical properties of the montmorillonites. *J. Pharm. Sci*. 55, 11 (1966): 1215-1220 [Wiley][Abstract]

16. Cheng-Che Tsai, Tzong-Yuan Juang, Shenghong A. Dai, Tzong-Ming Wu, Wen-Chiung Su, Ying-Ling Liu, Ru-Jong Jeng. Synthesis and montmorillonite-intercalated behavior of dendritic surfactants. *Journal of Material chemistry*. 16 (2006): 2056-2063 [Rsc][Astract].

17. T. Grygar, D. Hradil, P. Bezdieka, B. Doušová, L. Eapek, O. Schneeweiss. Fe(III)-modified montmorillonite and bentonite: synthesis, chemical and UV-Vis spectral characterization, arsenic sorption, and catalysis of oxidative dehydrogenation of propane. *Clays and Clay Minerals*. 55, 2 (2007): 165-176 [Geoscienceworld][Abstract].

18. J.A. Kittrick. Montmorillonite equilibria and the weathering environment. Soil Science Society of America Proceedings 35 (1971): 815-820 [Dl][Abstract].

19. I.A. Afonina. Effect of copper and zinc on the productive and biological indicators of laying hens cross «Rhodonite»: Dis ... Candidate. biol. Science. Novosibirsk State Agrarian University Institute of Veterinary Genetics and Breeding. Novosibirsk (2006): 208 p. (In Russian) [dissercat][Full text].

20. P. Hirsch, A. Hovi, R. Nicholson, D. Peshli, M. Whelan. Electronic microscopy of thin crystals. World (1968): 575 (In Russian) [Twirpx][Full text].

21. Methodical recommendations of MP 1.2.2641-10 «Determination of priority types of nanomaterials in environment objects, foodstuff and live organisms». (2010): 85 (In Russian) [RPN][Full text].

22. Methodical recommendations of MP 1.2.0022-11 «Sampling order for control of nanomaterials». (2011): 39 (In Russian) [Consultant][Full text].

23. K. Q. Nam, N. H. Toan Phan. Use of bentonite and diatomaceous earth for treatment of municMIAl and animal waste . *J.of Earth Sciences*. 24, 4, (2005): 351-355 [Sciencedirect][Full text].

24. Y. F. Cheng, Y. P. Chen, X. H. Li, W. L. Yang, C. Wen, Y. M. Zhou. Effects of Palygorskite Inclusion on the Growth Performance, Meat Quality, Antioxidant Ability, and Mineral Element Content of Broilers. *Biol Trace Elem Res* 173, 1 (2016): 194-201 [Springer][Full text].