THEORETICAL AND EXPERIMENTAL JUSTIFICATION FOR RESOURCE-SAVING AGRICULTURAL TECHNOLOGY IN HORTICULTURE

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Abstract

A theoretical basis for the natural minerals applying in agro-technologies based on the scientific papers analysis has been stated. The structural features and the chemical properties of Khotynetz zeolite have been shown on the large experimental data of the representative techniques. The results of agro-environment experiments have been listed. The samples morphological description has been given. The structure has been visualized and the characteristic pore sizes have been determined. The data on the chemical and analytical properties of Khotynetz zeolite were received. The order and the percentage of 10 major elements have been determined. The results of the agro-ecological experiments are given. The positive impact on the mineral plant biochemical indicators has been stated.

Key words: *zeolite, scanning electron microscopy, energy microanalysis, elemental composition, leaf, fruit, gooseberries, strawberries, morphology*

1. INTRODUCTION

The problem of soil pollution and obtaining of the safe production in the conditions of techno- and agrogenic pollution of the environment is urgent at present. In the aspect of the efficiency improving of fruit crops cultivation and food security the using of the nanoporous natural minerals to toxins neutralization in the soil is a new tool for the development of environment-saving agricultural technologies. Objective data obtained by Russian scientists - Belitsky I. A. and Panin L. E. (1990); Tarasevich, Y. I., Ovcharenko F. D., (1975); Popesco I. G. et al. (1991); Mayanskaya N. N., Novoselov Ya. B. (2000); Bgatova N. P. (2000); Leonteva L. I. (2008); Kuznetsov M. N., (2009); Ignatov I., Mosin O. V., (2014) and foreign researchers - Leppert, D., (1990); Virakornphanich P. and Egashira K., (1998); Zorpas Antonis A., Vlyssides Apostolos G. (1999); Derome J., (2000); Ponizovskii A. A., Dimoyanis D. D., Tsadilas K. D. (2003) prove the effectiveness of the natural zeolites agricultural usage. The zeolites have been already used in all developed countries of the world in the most significant areas of human activity (industry, defence, information technologies, energy, transport, biotechnology, medicine). The analysis of the investment growth and the introduction rate of the fundamental search engine developments allows to conclude that the usage of the nanotechnology and nanomaterials will be one of the determining factors of the scientific and economic development of states in the next 20 years. This direction is supported by the state and corresponds to the innovative development of Russia. In Orel region there is a Khotynetz deposit where the located reserves of zeolite are tens of mln.m3. 34% of clinoptilolite and 12% of montmorillonite form a part of the Khotynetz zeolite. Clinoptilolite is of special value as it is a highly siliceous mineral and has nano-canals and contents up to 40% of amorphous silicon. Montmorillonite is a schistous clayey mineral and has high absorption features. Such a mineralogical composition is a unique feature of the Khotynetz zeolite as a natural system with nano-features (http://www.zeomix.ru/propertiesofnaturalzeolites.html).

Therefore, study of the natural minerals structural and technological properties with the usage of the modern methods for the agricultural technologies introduction is urgent.

2. RESEARCH PURPOSE

The comprehensive study of the Khotynetz zeolite (Orel region, Russia) and the evaluation of its detoxifying activity in the cultivation of gooseberries and strawberries.

3. RESEARCH TASKS

3.1. Investigation of the mineral particles surfaces structure with a scanning electron microscope.

3.2. The determination of the mineral elemental composition.

3.3. The study of mineral sorption properties in lead ion relation.

3.4. The usage of zeolite as a soil improver in agro-ecological experiments in growing gooseberries and strawberries.

3.5. The change of the morphological and biochemical indices of strawberry plants photosynthetic organs while adding zeolite into the soil

3.6. The evaluation of the zeolite influence on the lead intake reduction in fruit.

4. MATERIALS AND METHODS

The objects of the investigation were air-dry samples of zeolite from Khotynetz deposit with granules of 0,5-0,2 mm in size, roundish form, grey color and insoluble in water. The morphology of the particles surface was studied by scanning electron microscopy (SEM) in conditions of secondary electrons with work tension 15-20 kW. The chemical composition of the zeolite – basic petrogenic elements (Si, Al, Ti, Fe, Mg, Ca, K, P, S) was determined by local energetic dispersion spectrometry (EDS). The locality of the analysis was 3 um, the sections not less than 12 um were scanned.

Relative errors of the chemical analysis were the following: with the element content from 1 up to 5% - not more than 10%; with the content from 5 up to 10% - not more than 5%; with the content over 10% - not more than 2%. 500 particles of zeolite were investigated. The morphometric analysis was performed using the program STIMAN (Sokolov et al., 1998).

The determination of absorption parameters of cations Pb2+ was carried out in the model solutions containing cations in concentrations 6 and 12 mg/l under a constant temperature T=293° K and different pH (5,6 and 7). The concentrations of Pb2+ enter the interval of concentrations of mobile forms of metals under different rates of the soil pollution. The speed of absorption of metal cations was studied in the static conditions under the relation of the liquid phase volume to the solid phase mass, V/m=100dm3•kg-1. The absorption process was being studied under the constant mixing and with the speed of 400 revolutions per minute. After certain time intervals (0,5; 1; 1,5; 2; 4; 6; 24 hours) the investigated solution was taken in fives ml and centrifuged with 5 000 revolutions per minute during 5 minutes. The lead content was determined by HLPC (Methods, 1996).

In field experiments with the zeolite intake the meliorant influence on the chemical composition of fruits and morphological and biochemical indices of gooseberry leaves (3 forms) and strawberries (3 varieties) were studied. In the pilot area of the soil the lead levels are 1.2 times higher than the permissible level. While gooseberries growing the zeolite was added at the rate of 24 tons per hectare. While strawberries growing - 15 tons per hectare. The lead content in the fruits was also determined by liquid chromatography (Motyleva, 2009). Ash leaves composition - by EDS. The morphology of leaves was examined by SEM.

5. RESULTS AND DISCUSSION

5.1. The visualization of Khotynetz deposit surface

The multicomponent composition of the samples was determined. The mineral bulk consists of the minerals from the plate, isometric, scaly, loose and porous structure. These minerals are from montmorillonite, mica, kaolinite and clinoptilolite group. The representative samples pictures are presented in Fig 1 - 4.

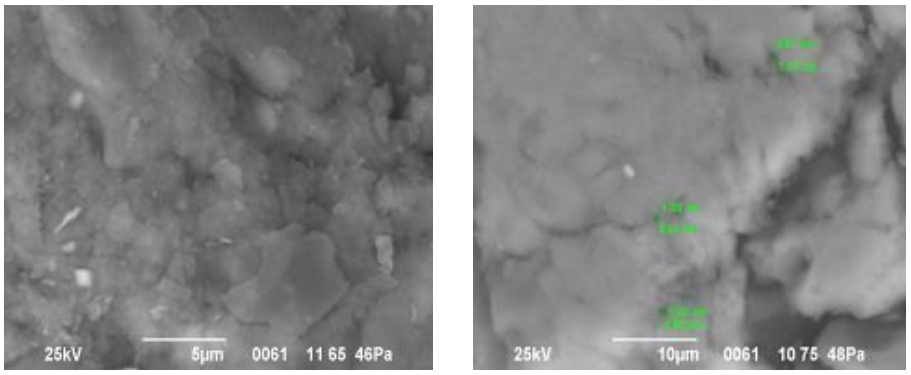


Fig. 1. Sample 1. The surface is loose, with inclusions of calcium, the average size of the micropores of 1.8 - 0.64 nm

Sample 1 contains aggregated particles with clear pin; large aggregates have less clear contours. There are micro-particles of kaolinite and montmorillonite and calcite impurities. There are a lot of very thin plates with rounded edges. Organic inclusions have not been identified.

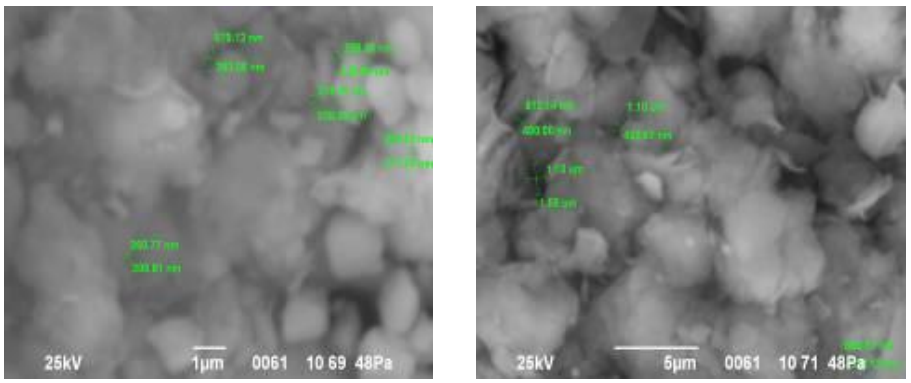


Fig. 2. Sample 2. The particles are layered with jagged edges.

In sample 2 silicates and calcite in the form of electron-dense structure are present. The particles are layered while zooming of more than 10,000 look loosely. There are macropores of 240 - 670 nm, round, irregular or slit-like shape.

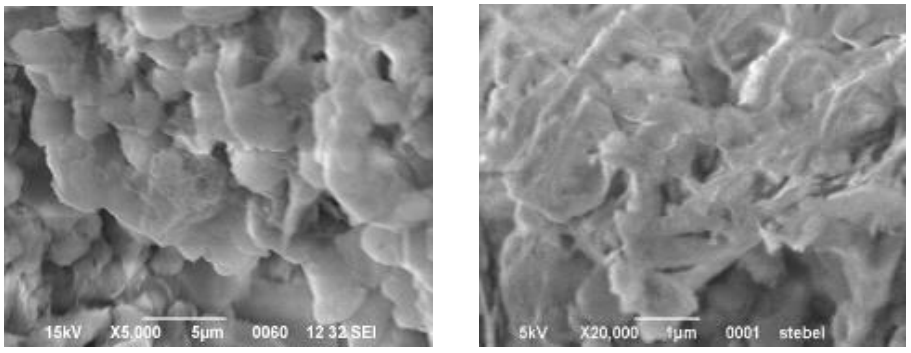


Fig. 3. Sample 3. Polydispersity fraction

The mineral particles of the sample 3 are loose, with porous structure. The size of the micropores is 1070-900 nm.

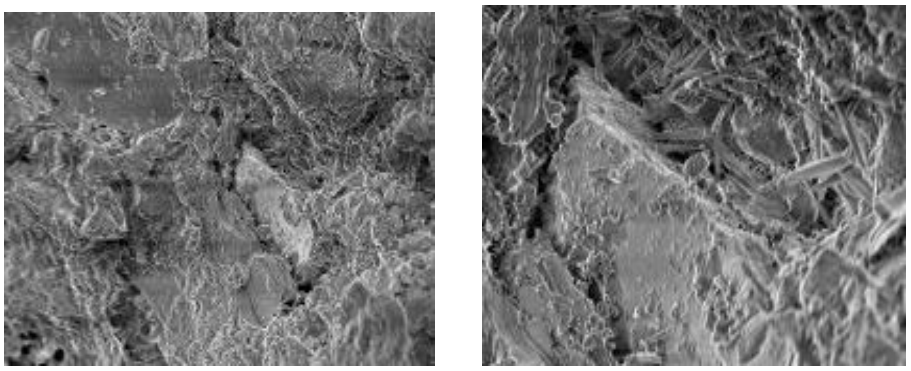
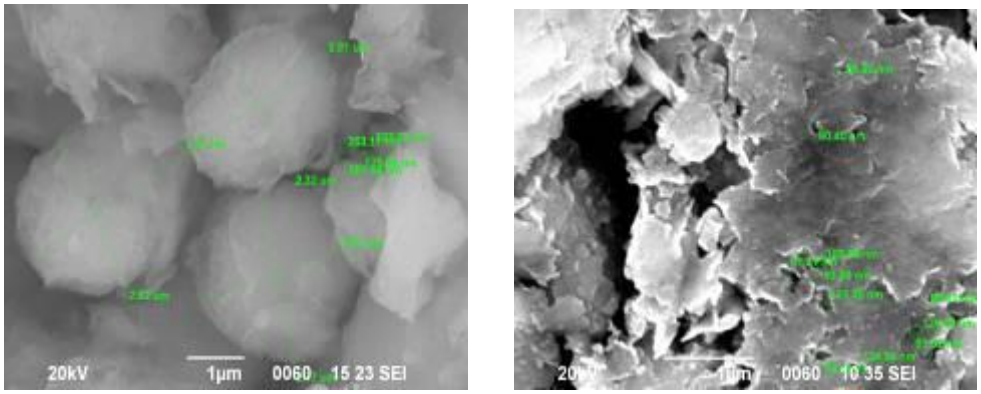


Fig. 4. Sample 4 has a lamellar structure

The long particle of sample 4 corresponds to kaolinite. Kaolinite particles are of pronounced pseudogonal form. The size of the mineral particles is from 200-400 nm to 25 um in length. Comparative pictures of the particles (0,05-0,02 mm), montmorillonite and kaolinita are shown in Fig.5. Montmorillonite forms layered, rounded particles. They fit snugly to each other. The particle diameter is 3 um. Clinoptilolite surface is smoother with a lot of 80-122 nm pores.



a **b**

Fig. 5. Microsculpture of montmorillonite (a) and clinoptilolite (b)

Electron microscopy studies confirmed the presence of many naturally formed close-up (> 200 nm) and micropores (from 0,6 - 07 to 1.2 - 1.6 nm). The structure morphological analysis is performed using the software "STIMAN '. The pore space analysis allows to determine the size of the total porosity. Total porosity is evaluated as the ratio of the total pore area S to a frame size with zooming Sfr:

$n = S / Sfr$

To determine this parameter the image analysis was used (Figure 6).

The pore perimeter P allows to determine such an important characteristic of natural mineral disperse samples as the specific surface area (So).

$So = P / Sfr$

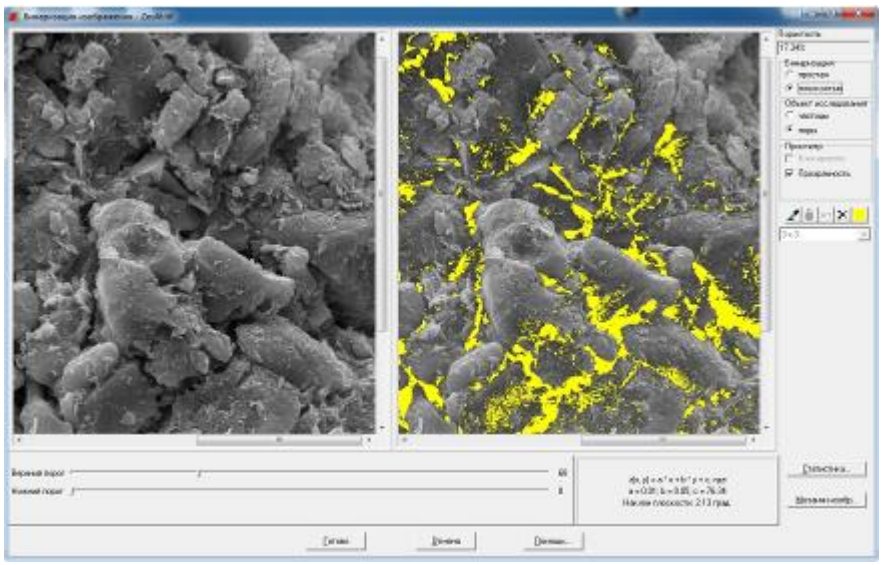


Fig. 6. Example of determining the pore space using “STIMAN” program

Since most of the samples have a contact porous polydisperse composition, the crucial in determining the size of the structural elements is their distribution areas, perimeters, equivalent diameter and total pore area (Table 1).

Table 1. The results of the morphometric analysis of samples (mean values)

Parameter	**Value**
The number of pores	324203
Porosity(%)	40, 2723
The total area of pores (kv.mkm)	652786,6280
The total perimeter of the pores (um)	415901,3672
Mean diameter (um) / dispersion	0,165-95/0,2277
The average size (kv.mkm) / variance	0,2014/156,9891
The average circumference (m) / variance	1,1903/28,8211
Specific surface (1 / mm)	1,2200

The analysis of the zeolite samples representative surface in three dimensions confirmed the presence of a complex pore system (Fig. 7).

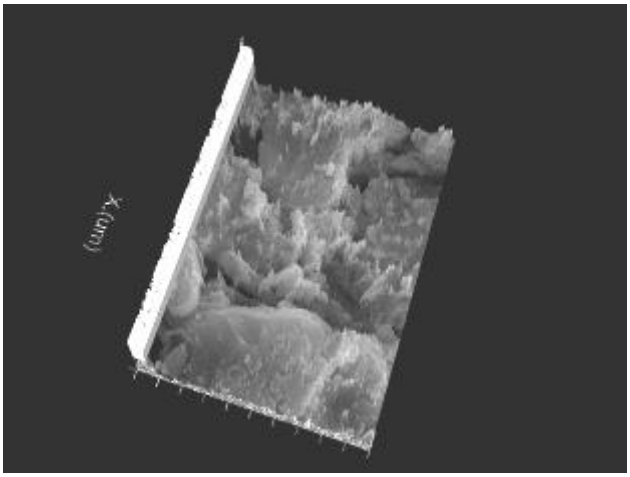


Fig. 7. A three-dimensional image of the zeolite surface using “STIMAN” program

We received the unique information about the features of the surface morphology and multicomponent composition of Khotynetz deposit samples. The main part consists of aggregate minerals with plate, isometric, scaly, loose porous structure (montmorillonite group minerals, mica, kaolin, clinoptilolite). These results are consistent with the heterogeneity of the internal structure of zeolite rocks associated with geomorphological cycles of continental territory and climate change (Chikishev A.G.,1979).

A comprehensive study of Khotynetz deposit samples surface morphology and morphometric analysis confirmed that it is able to exhibit a high adsorption capacity.

5.2. The major elements content in the zeolite samples

Via EDS method the features of the samples elemental composition are studied (Table 2). The differences between the fractions chemical composition are marked by the share of K and S. Si mass share is 22 - 40% on average. The average content of major elements in the samples is slightly different which highlights the relative homogeneity of Khotynetz deposit.

Table 2. The main elements that form the zeolite samples structure of Khotynetz deposit, % by weight.

Elements	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
O	48.59	41.29	49.38	49.26	47.75
Mg	0.61	1.66	3.66	0.81	0.68
Al	12.9992	6.62	6.86	13.06	7.93
Si	39.94	22.59	34.41	30.71	27.12
P	0.08	0.37	0.51	0.18	0.13
S	0.12	1.44	0.93	0.17	0.08
Ca	0.59	5.62	4.33	0.47	1.19
K	0.67	3.14	3.26	1.97	1.27
Ti	0.62	0.92	1.08	0.71	0.78
Fe	2.49	5.53	4.56	2.83	8.26

The order of test elements content is the following: Si> Al> Fe> K> Mg> Ca> Ti> S> P.

Toxic elements were not found.

5.3 Physico-chemical properties of Khotynetz deposit

Montmorillonite and clinoptilolite cause high adsorption properties of the mineral. Specific capacity is from 4500 to 6000 sm^2 /g. The exchange capacity for Na is 10 mg-eq / g at pH 7. The dispersion increases the specific capacity and exchange significantly. Within the scope of macro - micro and meso pores the main absorption is done by van der Waals forces of non-specific interaction and exactly their presence will affect the filtration and transport properties of Khotynetz deposit. In the metal detoxification and other harmful substances the sorption process that underlies in the mechanism of natural minerals action plays an important role (Blois M.S., 1958; Zhabin, Dmitriev, 2002; Karmosh, Alyabiev, Pogorelov, 2011). It has been shown experimentally that the adsorption of Pb-ions in the investigated concentrations depends on the pH and poorly depends on the concentration of ions in the solution. The quantitative sorption of ions Pb^{2+} (Fig. 8) occurs the most efficiently (60-80%) at pH 2,5 - 6. Samples 1,3,4 and 5 at these pH sorb 60 to 80% of Pb ions from the solution. Pb ions sorption in Sample 2 occurs most efficiently at pH 4-6.

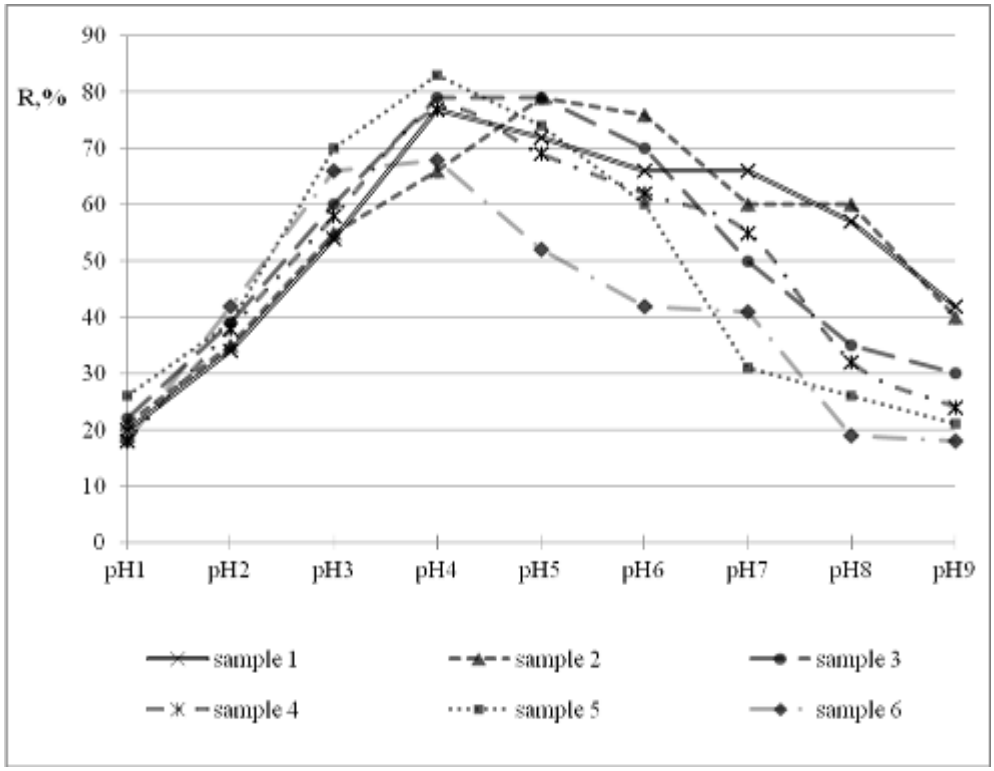
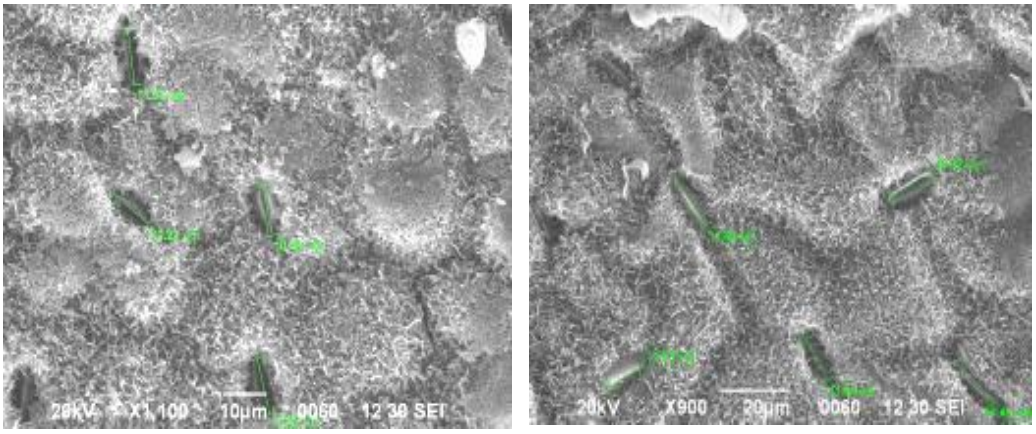


Fig. 8. The effect of pH (horizontal axis) on $Pb2^{+}$ ions recovery rate R,% (vertical axis)

R,%, characterizes the effectiveness of the sorbent and depends on the environment pH, the sorbed element and the sorbent (the zeolite sample). Macropores do not play a significant role in the static adsorption, but they influence the kinetics process significantly serving as a vehicle for long adsorbed cations (Dubinin, 1972). The zeolite pore effective size commensurate with the sorbed cations effective dimensions. The sorption flows through ion and the mixed-diffusion mechanisms.

5.4. Response reaction of gooseberries and strawberries leaves on adding the zeolite into the soil

The plant leaf is the main organ of photosynthesis and its response to stress factors is a natural (Honda, 1971). The confirmed positive impact of Khotynetz deposit zeolite on the leaves morphological indicators is proved. The average size of the gooseberry leaf upper epidermis cells on the plots with added zeolite is on 15 6 - 18.6 mm more than on the control ones. For the strawberry plants differences are 11, 6 - 17.4 mm. The gooseberry leaf stomata length on the control plots is 2.1 - 2.5 mm less than on the zeolite ones, for the strawberry leaves - 1.91 - 4.97 um. The breed-specific features of the strawberry leaves lower epidermis are clearly illustrated in Figures 9 - 11.

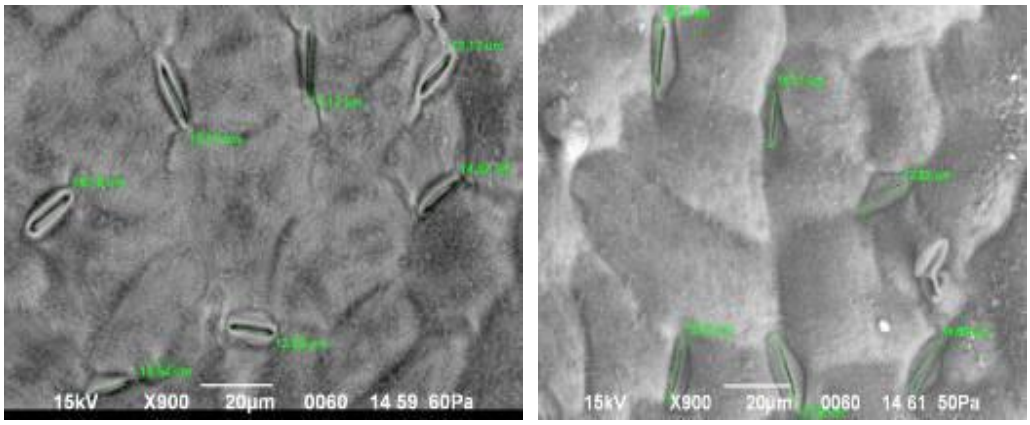


1 **2**

Fig. 9. The comparative size of the stomata. Mamochka breed strawberry leaf.

1 - the control plots, 2 - the zeolite ones

Mamochka breed leaves have a strong, waxy plated layer. The epidermal cells are convex, embossed. The stomata are located deep in the folds. The average size of the stomata on the control plots is 10.98 um. On the zeolite ones it is 15,95 um. The differences are 4.97 um.

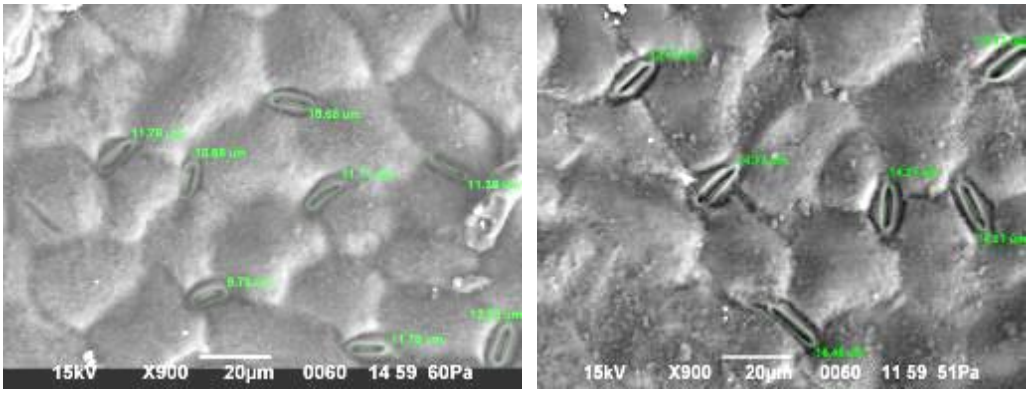


1 **2**

Fig. 10. The comparative size of the stomata. Bylinnaya breed strawberry leaf.

1 - the control plots, 2 - the zeolite ones

The wax layer of Bylinnaya breed leaf is less developed, its shape is slightly lumpy. On the control plots the average length of stomata is 15.64 um, on the zeolite ones it is 17.51 um; the differences are 1,97 um.



1 **2**

Fig. 11. The comparative size of the stomata. strawberry leaf.

1 - the control plots, 2 - the zeolite ones

In Rybinovyj kulon breed leaves the average length of stomata on the control plots is 11.2 um. On the zeolite ones it is 16.1 um. The differences are 4.91 um. The gooseberries and strawberries leaves stomata length on the control plots is on 2.1 - 2.5 mm less than on the zeolite ones. A statistically significant reduction of Pb and increased value of K and P was determined in the leaves on the plots with added zeolite. (Fig. 12).

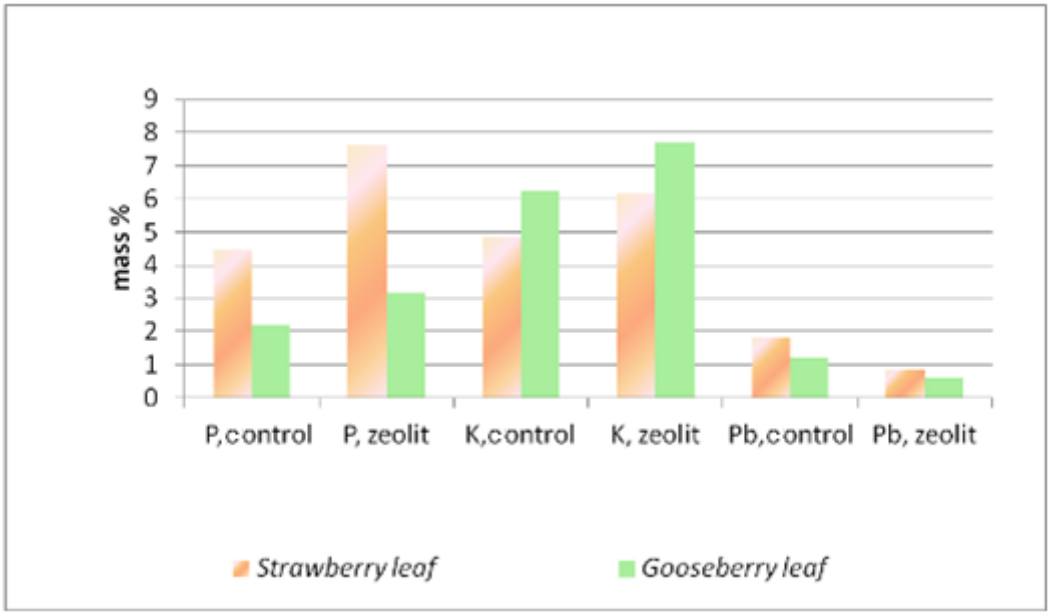


Figure 12. The changing of P, K and Pb content in the plants leaves

The results indicate the optimization of the plants nutrition when adding zeolite. The varietal characteristics of gooseberry and strawberries plants response on the growing conditions are identified. These differences are the most clearly seen in strawberry plants.

5.5. The elements content in the fruit

It has been established that adding of Khotynetz deposit zeolite at planting gooseberries and strawberries minimize intake of heavy metals in fruits (Fig. 13).

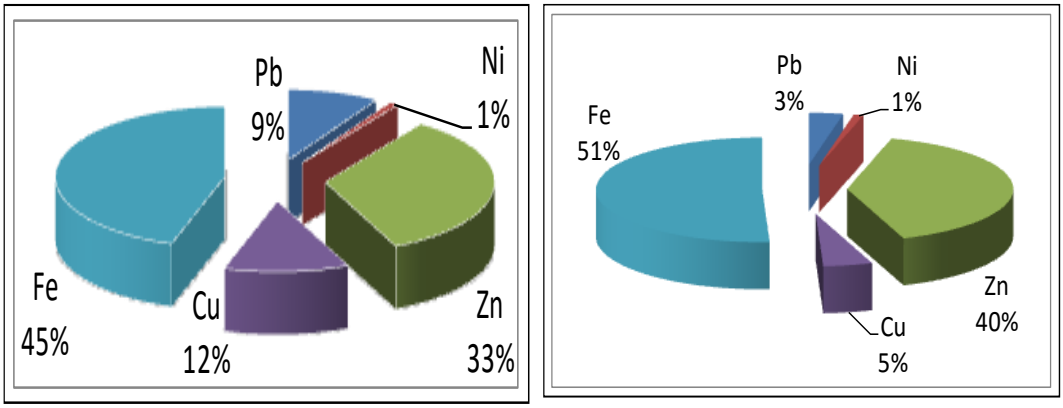


Fig. 13. The effect of zeolite on reducing of Pb intake in the gooseberry fruit.

Left – the control plots, right - the zeolite plot.

A positive effect of the zeolite on the morpho-anatomical parameters of photosynthetic organs of plants has also been noted. Our research is confirmed by the patent RU 2409925.

6. CONCLUSIONS

6.1. With the use of modern analytical equipment an in-depth study of Khotynetz deposit zeolite was performed. The features of the typical zeolite samples structure were identified. The quantitative morphological parameters of the microstructure are determined. The size, shape and orientation of the structural elements are described. The analysis of pore space is performed.

6.2. The major elements content in the samples is determined and the order of their accumulation is identified.

6.3. The regularities and selectivity of cation Pb2 adsorption from model solutions by different zeolites samples are identified.

6.4. A scientific basis for the development of application-based approach for the development of natural mineral methods of plant products protection from contamination with heavy metals is established.

6.5. Agro-field experiments with the addition of Khotynetz deposit zeolite into the soil once before gooseberries and strawberries planting were conducted. The positive effect of zeolite on the leaf morpho-anatomical indicators was determined. The improvement of the photosynthetic apparatus was revealed. The K and P intake is optimized, and the Pb intake in gooseberry and strawberry leaves is decreased.

6.6. The usage of Khotynetz deposit zeolite provides ecological safety of the fruit. When growing in the polluted areas Pb content in the gooseberry and strawberry fruit decreases 3 times on average.

REFERENCES

Belitsky I. A. and Panin L.E. (1990), 'The mineralogical and physical and chemical properties and biological activity of the zeolite-containing rocks', *Physico-chemical, medical-biological properties of natural zeolites*, Novosibirsk, P. 5-13.

Bgatova N.P. (2000), 'The use of biologically active food additives on the basis of natural minerals for the detoxification of the body', *Novosibirsk*, pp. 240.

Blois M.S., (1958), 'Antioxidant determinations by the use of a stable free radical', *Nature*, vol. 181, no. 4617, pp. 1199–2000.

Chikishev A.G., (1979), '*The study of Russian karst plain*', M., MGU.

Derome J., (2000). 'Detoxification and amelioration of heavy metal contaminated forest soils by means of liming and fertilization', *Environ. Pollut*. Vol. 107, No1. – P. 79-88.

Dubinin M.M., (1972), '*Adsorption and porosity*', M., VAHZ. -. p.126

Honda S.I. et al. Interpretation on chloroplast reproduction derived from correlation between cells and chloroplasts. Planta (Berl.), 97, 1971: 1-15)

Ignatov I., Mosin O. V., (2014). '*The composition and structural properties of fullerene-containing natural mineral shungite. Mathematical model of interaction of shungite with water molecules*', Internet Journal "Naukovedenie", issue 2, March - April 2014, p. 17, viewed 09 February 2016,< http://naukovedenie.ru/>.

Karmosh E.V., Alyabiev T.M., Pogorelov A.G. (2011), 'Chemical and mineralogical aspects of the possibil Karmosh E.V., Alyabiev T.M., Pogorelov A.G. (2011), ities of using clay Belgorod region to develop sorbents for the purification of waste water', *Basic Research*., vol. 8, pp. 131-136

Kuznetsov M.N., (2009), 'Zeolite effect on heavy metal content in grey forest soil in conditions of technogenic pollution', *Russian Agricultural Sciences.*, Vol. 35.–№3.–P. 179-181.

Kuznetsov M.N., Roeva T. A., Leonicheva E.V, Motyleva S.M. (2010), 'Influence of agro background on the effectiveness of the zeolite as a heavy metal deactivator in berry agrocenosis', *Contemporary Horticulture*, № 1.

Kuznetsov M.N., Roeva T. A., Leonicheva E.V., Motyleva S.M. 2011, *The method of reducing the heavy metal content in the berries of black currant*, RU Patent 2409925

Leonteva L.I. (2008), 'The effectiveness of the zeolite when grown Rúbus idáeus and Ribes uva-crispa L', synopsis of a thesis PhD of agricultural science, Orel, p. 23.

Leppert, D., (1990), 'Heavy metal sorption with clinoptilolite zeolite: alternatives for treating contaminated soil and water', *Mining Engineering* , №42, pp. 604–608

Mayanskaya N. N., Novoselov Ya. B. (2000), 'Sanogenetische principles of funds, which have been prepared on the basis of natural minerals (Russian)' *Ekor, Novosibirsk*, p. 1-85

Motyleva S.M., Kuznetsov M.N. (2009), 'Guidelines for determination of Pb and Zn in fruit plant organs', Orel, pub. GNU VNIISPK., p.23

MUK 4.1.053-96, (1996), 'Method of determination of heavy metals (Cd, Pb, Zn, Cu) in food products, food and feed environments model in extracts from taro, packaging materials with high performance liquid chromatography' - Moscow, State Committee.

Ponizovskii A.A., Dimoyanis D.D., Tsadilas K.D. (2003), 'The use of zeolite to detoxify soils contaminated with lead', *Eurasian Soil Science*, No 4. –p. 487-492.

Popesco I.G. et al. (1991), 'The study of the efficiency of the use of zeolites for reducing the fitotoxicity of heavy metals on wild strawberry, black currants and cherries', *The use of natural zeolites in the national economy. Part 1.*, Novosibirsk, P. 188-198.

Sokolov B.N., Yurkovets D.I., Razgulina O.V., Melnik V.N., (1998) 'Using Fourier analysis of SEM images for morphologic characteristics microsculpture', Proceedings of the Academy of Science, ser. phiz. №3, pp. 450-454.

Tarasevich, Y.I., Ovcharenko F,D., (1975), ' Adsorption on clay minerals' *Kiev Naukova Dumka*,.-S. 351.

Virakornphanich P. and Egashira K., (1998), 'Synthesis of Zeolite from Waste Lignite Ash from Thailand',*Soil Sci. Plant Nu tr.* , 44 (4), 707-710, 1998

Zhabin A.V, Dmitriev D.A. (2002), 'Authigenic mineral formation in the Upper Cretaceous and Paleogene deposits of the Voronezh anticline', *Herald Voronezh.un-ta. Ser. Geological Survey*, № 1, S. 84 – 94.

Zorpas Antonis A., Vlyssides Apostolos G. (1999),'Dewatered anaerobically –stabillzed primary sewage sludge composting: metal leachability and uptake by natural clinoptilolite', *Com. Soil Sci. and P1ant. Anal.*, Vol. 30, No11-12., P. 1603-1613.

"Zeolite technology" 2010 properties of natural zeolites, GC "Zeolite technology" viewed January 26, 2016, <http://zeomix.ru/propertiesofnaturalzeolites.html>